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REVIEW ARTICLE

Nitrogen metabolism in liver: structural and functional organization and physiological relevance*

Dieter HÄUSSINGER
Medizinische Universitätsklinik, Hugstetterstrasse 55, D-7800 Freiburg, Federal Republic of Germany

INTRODUCTION

The liver was identified early in the fundamental work of Hans Krebs [1,2] as having a particular role in urea and glutamine metabolism. At the acinar level, these pathways are embedded into a sophisticated structural and functional organization with metabolic interactions between different hepatocyte populations. This provided a new insight into the role of the liver in maintaining ammonia† and bicarbonate homeostasis under physiological and pathological conditions.

The functional units of the liver are the so-called acini [3,4], which extend from the terminal portal venule along the sinusoids to the terminal hepatic venule. Periportal hepatocytes (near the sinusoidal inflow) can be distinguished from more downstream located perivenous hepatocytes (near the sinusoidal outflow), whereas the borderline between the periportal and the perivenous compartment is not defined in general anatomical terms. The definition used here is a comparative-functional one, and thus will depend on the metabolic pathways under consideration. This is because periportal and perivenous hepatocytes differ in their complement of enzymes and their metabolic functions ('functional hepatocyte heterogeneity' or 'metabolic zonation'). The size of a periportal or perivenous 'metabolic zone' is pathway-specific. Thus, for example, a periportal compartment exhibiting a certain metabolic function can overlap with a perivenous compartment which is characterized by another pathway. Several reviews have appeared on this subject [5–11], and the subacinar localization of various metabolic pathways is summarized in Table 1. This article focuses on the functional significance of liver parenchymal cell heterogeneity in nitrogen metabolism.

ACINAR ORGANIZATION OF NITROGEN-METABOLIZING PATHWAYS

Different techniques (for recent reviews see [5,9,10]) have been employed to study metabolic zonation in hepatic nitrogen metabolism: histochemistry [12], immunohistochemistry [13–17], *in situ* hybridization [18,19], retrograde/antegrade liver perfusion [20,21], microdissection [22], autoradiography (B. Stoll, H. P. Buscher & D. Häussinger, unpublished work), radiolabel-incorporation studies [23,24], studies on zonal cell damage [25,26], use of micro-lightguides and mini-oxygen electrodes [27], attempts to separate periportal from perivenous hepatocytes [28–30] and the dual-digitonin-pulse perfusion [31]. These studies revealed a remarkable development of functional hepatocyte heterogeneity with respect to ammonia and glutamine metabolism. In the intact liver acinus, urea synthesis and glutaminase are present in periportal hepatocytes, whereas glutamine synthetase is found only in perivenous hepatocytes. This was shown in the structurally and metabolically intact perfused rat liver by comparison of metabolic fluxes during antegrade and retrograde perfusion [20], in experiments with zonal liver damage [25,26], by immunohistochemistry of carbamoyl phosphate synthetase [15], argininosuccinate synthetase [13], arginase [13] and glutamine synthetase [14] and more recently with hepatocyte preparations enriched in periportal and perivenous cells [28,29]. As shown by immunohistochemistry, the borderline between the periportal urea-synthesizing and the perivenous glutamine-synthesizing compartments is very strict [14,15]: glutamine synthetase is exclusively found in a small hepatocyte population (about 7% of all hepatocytes of an acinus) surrounding the terminal hepatic venule. These cells are virtually free of carbamoyl phosphate synthetase [15] and exhibit several further features which distinguish them clearly from cells of the much larger periportal compartment capable of urea synthesis. Some 70–100% of total hepatic glutamate uptake must be ascribed to this small perivenous cell population [21,24,30,32,33] and vascular oxoglutarate is taken up in a largely Na^+-dependent manner almost exclusively by perivenous glutamine synthetase-containing hepatocytes, but not by the much larger periportal cell population [24]. Also, plasma membrane transport systems are distributed heterogeneously among subacinar cell populations and the data provide an example for a common subacinar localization of functionally linked processes: glutamate and oxoglutarate are taken up almost exclusively by perivenous hepatocytes as a substrate for glutamine synthesis [21,23,24]. Carbonic anhydrase isoenzymes II and III [34], as well as a phenobarbital-inducible cytochrome *P*-450 isoenzyme [35] show an immunohistochemical localization almost identical to that of glutamine synthetase. On the other hand, mitochondrial carbonic anhydrase V [36] is periportal, as suggested by its functional linkage to urea synthesis and gluconeogenesis [36–41]. There is some evidence that perivenous glutamine synthetase-containing hepatocytes exhibit a higher proliferative activity *in vitro* than do periportal hepatocytes [42]. Whereas glutamate is almost exclusively taken up by perivenous hepatocytes [21,24], other amino acids such as proline, alanine, glutamine are taken up predominantly by periportal hepatocytes [20,21,30,32], i.e. into a compartment with high gluconeogenic and ureogenic capacity. In addition, periportal hepatocytes exhibit higher activities of amino acid metabolizing enzymes, such as alanine aminotransferase, tyrosine aminotransferase and glutaminase [20,43,44]. On the other hand, glutamate dehydrogenase is present in all hepatocytes. However, its activity shows a U-shaped gradient along the acinus with highest activities at the periportal and perivenous end of the acinus [16]. Glutamine transaminases are present in periportal as well as in perivenous hepatocytes [45].

* This article is dedicated to Professor Dr. Karl Decker on the occasion of his 65th birthday.

† Except when specified, ammonia refers to the sum of ammonium ions (NH_4^+) and ammonia free base (NH_3).

Table 1. Localization of metabolic pathways in the liver acinus

The size of the periportal and perivenous compartments, respectively, depends on the pathway under consideration. Gradient-type zonation: a metabolic function is present in all hepatocytes, but unequally distributed along the acinus with highest activities in either periportal or perivenous hepatocytes. Strict-type zonation: a metabolic function is restricted to a subacinar cell compartment, whereas it is not detectable in other subacinar compartments.

Periportal	Perivenous	Zonation type
Gluconeogenesis	Glycolysis	Gradient
Glycogen synthesis from lactate and amino acids	Glycogen synthesis from glucose	Gradient
Fatty acid oxidation	Fatty acid synthesis	Gradient
Urea synthesis	Glutamine synthesis	Strict
Glutaminase	Biotransformation	?/gradient and strict
Ketogenesis		
Amino acid uptake and degradation (except glutamate)		Gradient
Carbonic anhydrase V	Carbonic anhydrase II, III	?/strict
	Glutamate/oxoglutarate uptake	Strict

DYNAMICS OF THE COMPLEMENTARY DISTRIBUTION OF UREOGENESIS AND GLUTAMINE SYNTHESIS IN THE LIVER ACINUS

The reciprocal distribution of urea cycle enzymes and glutamine synthetase was shown for rat, mouse and human liver [13–15,46–48]. This organization seems unique for mammalian liver, whereas glutamine synthetase is homogeneously distributed in livers from uricotelic animals such as birds, reptiles and crocodilians [17]. A zonal distribution of carbamoyl phosphate synthetase and glutamine synthetase is also absent from tortoise liver, which possesses both uricotelic and ureotelic ammonia detoxifying mechanisms ([17]; for further aspects on comparative biochemistry see [49]).

Ontogenesis of zonal heterogeneity

The marked heterogeneity of glutamine and urea synthesis raises the interesting question of the factors determining this strict compartmentation. Hormone, oxygen and substrate gradients along the acinus were causally related to the development of hepatocyte heterogeneity in glucose metabolism [5,9–11], which is, however, characterized by more or less flat enzyme gradients along the acinus. This is in contrast to the strict reciprocal distribution of carbamoyl phosphate synthetase and glutamine synthetase (gradient-type versus strict-type zonation; see Table 1): cells contain either carbamoyl phosphate synthetase or glutamine synthetase, but both enzymes are normally not found within the same cell. Thus, acinar hormone and substrate gradients are probably not the sole determinants of metabolic zonation of nitrogen-metabolizing pathways. It recently became clear that the complementary distribution of carbamoyl phosphate synthetase and glutamine synthetase in the rat liver acinus is regulated at a pretranslational level [17–19]; the subacinar distribution of the respective mRNAs is identical with the distribution of the respective immunoreactive enzyme proteins. The ontogenesis of this enzymic zonation is developmentally regulated, and seems to be related to the development of hepatic acinar architecture rather than being a result of perinatal adaptation [50]. Although the heterogeneous distribution of carbamoyl phosphate synthetase coincides in time with the development of hepatic noradrenergic innervation, both events are probably not causally related [47]. It is interesting to speculate on the role of cell–cell communications or interactions with the extracellular matrix as determinants of the heterogeneous gene expression in the periportal urea-synthesizing and perivenous glutamine-synthesizing compartment. In line with this is the observation that all hepatocytes can be induced to express glutamine synthetase after transplantation into the environmental conditions present in fat pads [51].

In the rat, the reciprocal distribution of glutamine synthetase and carbamoyl phosphate synthetase develops rapidly after birth; whereas at birth carbamoyl phosphate synthetase is homogeneously distributed in the liver acinus, its heterogeneous distribution develops during the first 6 days post partum. On the other hand, the exclusive perivenous localization of glutamine synthetase is already present 2 days before birth [52,53]. The spiny mouse liver exhibits the adult pattern of the enzyme distribution at birth [50]. In human liver, the definitive architecture of the acinus is not yet complete at birth. In line with this, the ontogenesis of heterogeneity of ammonia-metabolizing enzymes has not developed 2 days after birth; it occurs thereafter and is mostly completed after 6 years, i.e. the first time point studied so far after the second postpartal day [48]. The compartmentation of carbamoyl phosphate synthetase and glutamine synthetase in the adult human liver is somewhat different to that in rat liver, in that a well-recognizable intermediate zone can be distinguished in human liver in which neither carbamoyl phosphate synthetase nor glutamine synthetase are detectable [48].

Dynamics of zonal heterogeneity

The heterogeneous distribution of carbamoyl phosphate synthetase and glutamine synthetase is remarkably stable. It is preserved during isolation of hepatocytes and persists in cultured cells for at least 3 days [14]. Perivenous glutamine synthetase-containing hepatocytes can selectively be destroyed by an appropriate dose of CCl_4. Such livers fail to synthesize glutamine and to take up glutamate, whereas urea formation from ammonia is not impaired [21,25,26]. No adaptive expression of glutamine synthetase occurs during prolonged CCl_4 intoxication and destruction of perivenous hepatocytes. Following recovery from a perivenous CCl_4 liver injury, glutamine synthetase recurs in the same acinar position as it is found in normal livers [26]. On the other hand, induction of a periportal liver cell necrosis by allylformate is accompanied by a marked loss of urea cycle enzyme activity, whereas glutamine synthetase activity remained unaffected [26]. No condition has been identified yet in which glutamine synthetase can be expressed in periportal urea-synthesizing hepatocytes or in which the perivenous cell population containing glutamine synthetase can be enlarged. Induction of glutamine synthetase by growth hormone and glucocorticoids

[53–55] increases the enzyme activity per cell, but does not increase the number of hepatocytes containing glutamine synthetase [46]. Only a few conditions were found where carbamoyl phosphate synthetase is coexpressed in perivenous glutamine synthetase-containing hepatocytes, such as glucocorticoid treatment in diabetes or in a short perinatal period [50,52,56], although carbamoyl phosphate synthetase activity was shown to underly marked fluctuations depending on the hormonal and nutritional state [56–59]; for review see [6,7]).

FUNCTIONAL SIGNIFICANCE OF HEPATOCYTE HETEROGENEITY IN NITROGEN METABOLISM

Glutamine synthetase acts as a scavenger for the ammonia escaping periportal urea synthesis

In the intact liver acinus, the two major ammonia-detoxicating systems, urea and glutamine synthesis, are anatomically aligned behind each other. Accordingly, the portal blood will first come into contact with hepatocytes capable of urea synthesis, before glutamine-synthesizing cells just at the end of the acinar bed are reached [20]. In functional terms this organization represents the sequence of a periportal low-affinity but high capacity system (ureogenesis) and a perivenous high-affinity system for ammonia detoxication (glutamine synthesis) [20,25,60]. In isolated perfused rat liver, efficient ammonia extraction with physiologically low portal ammonia concentrations requires an intact glutamine synthetase activity ('high-affinity system') and ammonia at physiological portal concentrations of 0.2–0.3 mM is converted by about two-thirds into urea and by about one-third into glutamine, although these pathways are structurally organized in sequence [20]. A 7% [^{13}N]ammonia recovery in glutamine was also shown *in vivo* following a bolus injection of [^{13}N]ammonia; this value may underestimate the real contribution of perivenous hepatocytes to hepatic ammonia clearance due to label dilution by endogenously formed ammonia in periportal cells [61]. These data suggest that *in vitro* and *in vivo* a considerable fraction of the ammonia delivered via the portal vein from the intestine reaches the perivenous end of the liver acinus. Here, perivenous glutamine synthetase acts as a high-affinity scavenger for the ammonia which escaped periportal detoxication by urea synthesis [20,24]. This also holds for ammonia produced during amino acid breakdown in the much larger periportal compartment; under these conditions ammonia is released from periportal cells into the sinusoidal space, despite the high urea cycle enzyme activity in this compartment [20]. This ammonia, however, is delivered via the blood stream to perivenous hepatocytes and is used for glutamine synthesis [20]. This not only underlines the comparatively low affinity of periportal urea synthesis for ammonia and the importance of ammonia scavenging by perivenous hepatocytes for efficient hepatic ammonia detoxication, but also demonstrates metabolic interactions between different subacinar cell populations [20]. Such a metabolic cell–cell interaction also implies different directions of flux through glutamate dehydrogenase in periportal and perivenous cells [62] respectively; periportal formation of NH_4^+ from amino acids requires oxidative deamination of glutamate, whereas perivenous NH_4^+ fixation for glutamine synthesis implies reductive amination of oxoglutarate. The difference in ammonia affinity between urea and glutamine synthesis results from the higher K_m of 1–2 mM [63] for NH_4^+ of carbamoyl phosphate synthetase, the rate-controlling enzyme of the urea cycle [64,65], compared with that for isolated glutamine synthetase (K_m 0.3 mM [66]). A similar difference in $K_{0.5}$ values of urea and glutamine synthesis from NH_4Cl is also observed in isolated perfused rat liver [21,67] and even more pronounced in human liver ($K_{0.5(NH_4^+)}$ for urea and glutamine synthesis 3.6 mM and 0.11 mM, respectively [68]).

The important scavenger role of perivenous glutamine synthesis for the maintenance of physiologically low ammonia concentrations in the hepatic vein becomes evident after inhibition of glutamine synthetase by methionine sulphoximine [20], or after destruction of perivenous cells by CCl_4 treatment [25]. In the latter case, hyperammonaemia ensues due to an almost complete failure to synthesize glutamine, although periportal urea synthesis is not affected [25]. In human liver cirrhosis the capacity to synthesize glutamine from ammonia is decreased by about 80% and a defective perivenous ammonia scavenging may contribute to the development of hyperammonaemia in liver cirrhosis [68]. When the acinar blood flow is experimentally reversed to the retrograde (i.e. from the hepatic to the portal vein), ammonia release from the liver increases about 8-fold despite unchanged rates of amino acid breakdown from endogenous sources [20]. This is because ammonia being released from periportal hepatocytes is washed out of the liver without coming into contact with cells capable of glutamine synthesis.

The capacity of perivenous cells to synthesize glutamine from NH_4^+ is remarkable. Highest rates of glutamine synthesis in perfused rat liver are about 0.6 μmol/min per g of liver [23], which corresponds to 8–10 μmol/min per g of perivenous cells. This is about 2-fold higher than the capacity of the same amount of periportal hepatocytes to eliminate ammonia via urea synthesis. The carbon skeleton required for glutamine synthesis is in part provided by an almost exclusive uptake of vascular 2-oxoglutarate [23], glutamate [21,24,32] and malate (B. Stoll & D. Häussinger, unpublished work) into these perivenous cells. It is likely, however, that the major part of 2-oxoglutarate required for glutamine synthesis is derived from glucose or lactate.

Recent data suggest that perivenous hepatocytes also play an important role in the inactivation of signal molecules such as extracellular nucleotides and eicosanoids, thereby extending their well-documented scavenger role for ammonia to a variety of other compounds [69,70]. This has led to the perivenous scavenger cell hypothesis [70], stating that one function of perivenous hepatocytes is to eliminate a variety of potentially toxic and harmful compounds just before the sinusoidal blood enters the systemic circulation. In the present context, the term perivenous scavenger cell is used synonymously with perivenous glutamine synthetase-containing hepatocytes.

With respect to the regulation of hepatic ammonia metabolism, four major implications arise from the structural and functional organization of hepatic ammonia metabolism. (i) With a constant portal ammonia supply, flux through the urea cycle will determine the amount of ammonia reaching the perivenous hepatocytes, and accordingly the substrate supply for glutamine synthetase. (ii) Periportal urea cycle flux can be varied without threat of hyperammonemia, because glutamine synthesis in perivenous scavenger cells acts as a 'back-up system' for ammonia detoxication, largely ensuring non-toxic ammonia levels in effluent hepatic venous blood even when urea cycle flux may decrease. Thus, control of urea synthesis can be uncoupled from the requirements of systemic ammonia homeostasis. (iii) Following the sinusoidal blood stream along the periportal compartment, an exponential decrease of the ammonia concentration is expected when ammonia is delivered via the portal vein in physiological concentrations. Accordingly, changes in portal ammonia supply may involve the recruitment of additional (more downstream) hepatocytes capable of urea synthesis [7]. (iv) An impairment of perivenous scavenger cells will lead to hyperammonaemia. This also occurs when the sinusoidal blood flow is reversed, because now the high affinity system for ammonia detoxication (glutamine

synthesis) is switched behind periportal urea synthesis ('low-affinity system of ammonia detoxication').

Glutaminase acts as a pH- and hormone-controlled mitochondrial ammonia amplification system

Whereas glutamine synthetase is perivenous, glutaminase is found in periportal hepatocytes [20,29] and has a joint mitochondrial localization together with carbamoyl phosphate synthetase. Phosphate-dependent glutaminase is the major enzyme involved in hepatic glutamine degradation, and is immunologically and kinetically different from glutaminases in other tissues (for reviews see [6,7,71–73]). The liver enzyme is not inhibited by its product glutamate [2], but requires its other product ammonia as essential activator [67,74–78]. In perfused rat liver halfmaximal activation of glutaminase occurs at the physiological portal ammonia concentration of 0.2–0.3 mmol/l and a maximal activation at 0.5–0.6 mmol/l [75,76]. Thus, fluctuations of the portal ammonia concentration in the physiological range are paralleled by changes in activity of hepatic glutaminase (so-called 'interorgan feed-forward' between intestinal ammonia production and hepatic glutamine breakdown [75]). In view of this unique product (ammonia) activation, the function of glutaminase is now seen to amplify ammonia inside the mitochondria in parallel to that delivered via the portal vein or arising during hepatic amino acid breakdown, although direct measurements of the actual ammonia concentration inside the mitochondria are still lacking. Because normally urea synthesis is controlled by flux through carbamoyl phosphate synthetase [64,65], which depends largely on the actual ammonia concentration inside the mitochondria, amplification of mitochondrial ammonia via glutaminase flux control becomes an important determinant of urea cycle flux. This is especially relevant in presence of physiologically low ammonia concentrations, which are about one order of magnitude below the $K_{m(ammonia)}$ of carbamoyl phosphate synthetase. Here, the low ammonia affinity of carbamoyl phosphate synthetase is counteracted by amplification of the operational ammonia concentration in the mitochondria and urea cycle flux is augmented [20]. In line with this, a 50% increase of urea synthesis is observed in perfused rat liver when glutamine (0.6 mmol/l) is added to the perfusate which already contains a physiological ammonia concentration [20]. Three further features add to the functional link between hepatic glutaminase and carbamoyl phosphate synthetase. (i) Both enzymes are activated by *N*-acetylglutamate [79]; thus this compound may play a role besides ammonia in coordinating the activity of both enzymes. (ii) There is some evidence for a channelling of glutaminase-derived ammonia into carbamoyl phosphate synthetase [80]. (iii) In contrast to ammonia being delivered via the portal vein, glutamine-derived ammonia is utilized for carbamoyl phosphate synthesis without control by mitochondrial carbonic anhydrase V [39].

As anticipated from a control of urea cycle flux by glutaminase activity, factors known to affect urea cycle flux are indeed associated with parallel activity changes of the 'mitochondrial ammonia amplifier' glutaminase. Apart from the portal ammonia concentration, this includes the effects of glucagon [74,75], α-adrenergic agonists [81–83], vasopressin [84], acidosis/alkalosis [76,85–87], feeding of a high protein diet [88,89] and liver cell volume changes. Liver swelling, as it occurs during active uptake of amino acids (which in turn stimulates amino acid breakdown), activates glutaminase and is followed by a volume-regulatory K^+ efflux [90,91]. Although these ion fluxes largely restore the liver cell volume within minutes, glutaminase remains in an activated state [91], possibly due to a prolonged mitochondrial swelling [92], and urea cycle flux is augmented [91]. Another important site controlling glutaminase flux (i.e. the activity of the ammonia amplifier) is glutamine transport across the plasma and the mitochondrial membrane ([45,93,94]; for reviews see [6,7,95]). Plasma membrane transport of glutamine occurs via the Na^+-dependent system N [96–99], whereas the glutamine transport system across the mitochondrial membrane is not yet characterized. These transport systems build up glutamine concentration gradients: at a physiological extracellular glutamine concentration of 0.6 mM, the cytosolic and mitochondrial concentrations are about 7 and 20 mM respectively in rat liver *in vivo* and *in vitro* [93,94]. Therefore, glutaminase operates under physiological conditions at glutamine concentrations near its $K_{m(glutamine)}$ of about 28 mM [71]. Control of the mitochondrial steady state glutamine concentration by the activity of glutamine transporters in turn regulates glutaminase flux and mitochondrial ammonia amplification. One example is transport control by pH; at a constant extracellular glutamine concentration of 0.6 mM, an increase of the extracellular pH from 7.3 to 7.7 increases the mitochondrial glutamine concentration from 15 to 50 mM [94]. This is paralleled by a 3–4-fold increase of flux through glutaminase and a stimulation of urea synthesis [76,85]. Apart from the mitochondrial/cytosolic proton concentration gradient, the activity of the Na^+/H^+ exchanger in the plasma membrane seems to be involved in the adjustment of subcellular glutamine concentrations, as suggested by the effect of amiloride on subcellular glutamine concentrations [100]. It is interesting to note that the activity of glutamine transport increases under conditions known to stimulate urea synthesis, such as dexamethasone treatment [101] and feeding of a high-protein diet [88], whereas it is not stimulated during short-term starvation [102].

Intercellular glutamine cycling

In the intact liver, periportal glutaminase and perivenous glutamine synthetase are simultaneously active, resulting in periportal breakdown and perivenous resynthesis of glutamine [20,62,75,76,82]. This was shown in perfused rat liver and later also demonstrated *in vivo* [61,103]. This energy-consuming cycling of glutamine is called the intercellular glutamine cycle [20]. In the presence of physiological glutamine and ammonia concentrations, flux through the glutamine cycle is 0.1–0.2 μmol/g per min. Glutamine cycling is under complex metabolic and hormonal control, involving flux changes through both periportal glutaminase and perivenous glutamine synthetase [60,62,75, 76,82,85]. This complex and simultaneous regulation of periportal breakdown and perivenous resynthesis of glutamine explains the controversial findings in earlier studies on the role of the liver in glutamine metabolism, which was either identified with net glutamine uptake or release or no net glutamine turnover at all [104–107].

With respect to hepatic nitrogen metabolism, intercellular glutamine cycling provides an effective means for adjusting ammonia flux into either urea or glutamine according the needs of the acid–base situation (see below). In the special case of a well-balanced acid–base situation, intercellular glutamine cycling allows maintenance of a high urea cycle flux, despite the low affinity of carbamoyl phosphate synthetase for ammonia [20] and the presence of physiologically low ammonia concentrations. This is achieved by periportal glutamine consumption during the mitochondrial ammonia amplifying process, which increases urea cycle flux, whereas periportally consumed glutamine is simultaneously resynthesized by perivenous scavenger cells from the ammonia which escaped the periportal compartment (Fig. 1). In perfused rat liver and with physiological ammonia and glutamine concentrations, up to 30% of the urea produced is due to periportal glutamine breakdown [20,60,85]. At normal extracellular pH, flux through glutaminase and glutamine synthetase

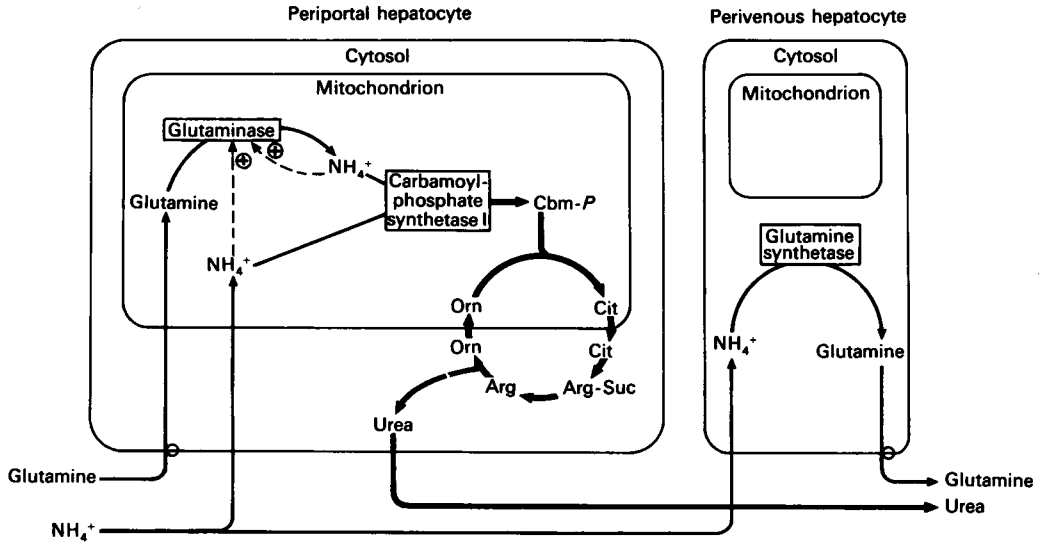

Fig. 1. Intercellular glutamine cycling and ureogenesis

Periportal glutaminase is activated by ammonia and acts as a pH- and hormone-modulated ammonia amplifier inside the mitochondria. The activity of this amplifier determines flux through the urea cycle ('low-affinity system for ammonia detoxication'). Glutamine synthetase in perivenous cells acts as scavenger for the ammonia escaping periportal urea synthesis ('high-affinity system for ammonia detoxication'). This anatomical sequence of low and high affinity detoxication systems uncouples urea synthesis from the primary need to maintain non-toxic ammonia levels and provides the basis for acid–base control of urea synthesis without threat of hyperammonaemia. A complete conversion of a portal ammonia load into urea occurs at a well balanced acid–base situation. Under these conditions periportal glutamine consumption (ammonia amplifying) and perivenous glutamine synthesis (ammonia scavenging) match each other: there is no net glutamine turnover by the liver, but portal ammonia is converted efficiently into urea despite the low ammonia affinity of carbamoyl phosphate synthetase. From [7].

is of equal magnitude. There is no net glutamine turnover, but glutamine cycling allows the almost complete conversion of a physiologically low portal ammonia load into urea. Flux through the intercellular glutamine cycle increases with the portal ammonia load and half-maximal flux through the cycle is observed at the physiological portal ammonia concentration of about 0.25 mM [76].

Intercellular glutamine cycling implies opposite net glutamine movements across the plasma membrane of periportal and perivenous hepatocytes, respectively. There is some evidence that periportal glutamine uptake occurs via Na^+-dependent system N [93,96,97], whereas release of newly synthesized glutamine from perivenous scavenger cells is Na^+-independent and involves facilitated diffusion [30,97]. Both transport systems are inhibited by histidine [93,96,97].

Glutamate release by the liver is normally very low, but is markedly stimulated by organic monocarboxylates such as oxoisocaproate, benzoate or oxomethionine [108,109]. Such an organic acid-stimulated glutamate export out of liver cells adds another site of control of hepatic ammonia metabolism [108,109]. Whereas vascular glutamate is taken up almost exclusively by perivenous scavenger cells [21,24], it is not yet clear whether glutamate exported under the influence of oxoisocaproate and benzoate also comes from periportal cells. Thus, the existence of an intra-acinar transfer of glutamate from periportal to perivenous hepatocytes [108] and its quantitative importance remains an interesting speculation.

Role in acid–base homeostasis

Acid–base homeostasis is traditionally seen to involve just two organs, the lungs and the kidneys. Recent conceptual developments, however, point to an involvement of the liver as a major pH homeostatic organ (for reviews, details and controversies see [110]). Such a hepatic role is based on three metabolic features. (i) The presence of a quantitatively important and liver-specific pathway for irreversible removal of metabolically generated bicarbonate, i.e. urea synthesis [111–116]. (ii) A sensitive and complex control of bicarbonate disposal via this pathway by the extracellular acid–base status [7,38,60,62,85,110,111,113, 115–117], which creates a feed-back control loop between the actual acid–base status and the rate of bicarbonate elimination, i.e. a bicarbonate-homeostatic response [7,38,85,110,116,117]. (iii) A structural-functional prerequisite, which uncouples urea synthesis from the vital need for maintenance of ammonia homeostasis and provides the basis for sensitive acid–base control of urea synthesis [20,60,62,85,110,116].

As pointed out by Atkinson and Bourke [111–115], catabolism of proteins generates large amounts of HCO_3^- and thus a threat of alkalosis. This is because oxidation of bipolar amino acids yields the strong base HCO_3^- together with the weak acid NH_4^+, as shown in the example of alanine oxidation:

$$2CH_3CH(NH_3^+)CO_2^- + 6O_2 \rightarrow 2HCO_3^- + 2NH_4^+ + 4CO_2 + 2H_2O$$

Taking into account the amino acid composition of an average protein diet (containing besides neutral amino acids also basic and acidic amino acids), the complete oxidation of proteins yields HCO_3^- and NH_4^+ in almost equimolar amounts. Elimination of this HCO_3^- (and NH_4^+) load derived from protein catabolism is a vital need for all living organisms and nature has developed different strategies. Whereas fish excrete HCO_3^- and NH_4^+ as such via their gills into the surrounding water, land-living species have developed pathways catalysing an irreversible proton transfer from NH_4^+ to HCO_3^-. This is urea synthesis in mammals and purine synthesis in birds, some reptiles and insects. For further developmental aspects and species differences in HCO_3^- and NH_4^+ disposing pathways the reader is referred to [49,112,114,118]. Thus, a major function of urea synthesis in mammals is the removal of bicarbonate, which is produced

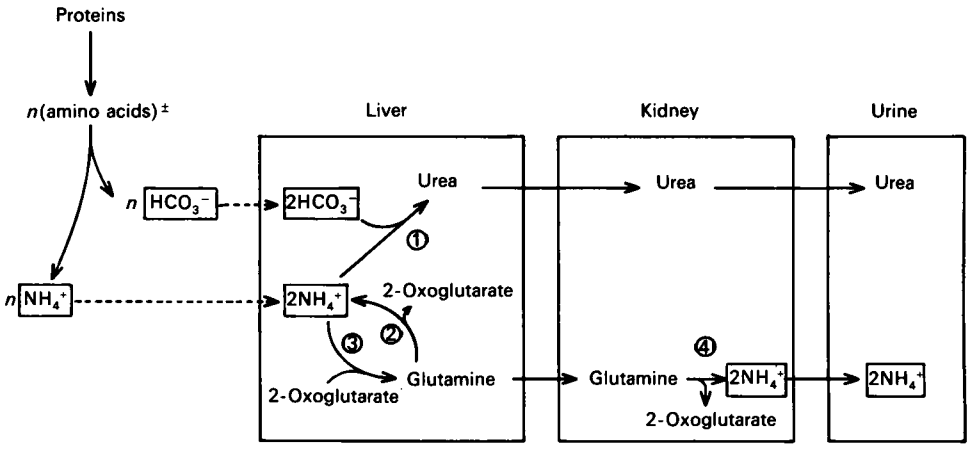

Fig. 2. NH_4^+ metabolism and HCO_3^- homeostasis

NH_4^+ and HCO_3^- generation are ultimately linked in a 1:1 stoichiometry during protein catabolism as is the irreversible elimination of both compounds via hepatic urea synthesis. Flux through the urea cycle is sensitively controlled by extracellular pH, $[HCO_3^-]$ and $[CO_2]$; these mechanisms adjust bicarbonate-consuming urea synthesis to the requirements of acid–base homeostasis. When urea synthesis decreases relative to the rate of protein catabolism in acidosis, bicarbonate is spared, and NH_4^+ is excreted as such into urine ('renal ammoniagenesis'), with glutamine serving as non-toxic transport form of NH_4^+ from liver to kidney. When NH_4^+ is excreted into urine, there is no net production or consumption of 2-oxoglutarate in the organism. Numbers in circles refer to major points of flux control by the acid–base status. In metabolic acidosis, flux through the urea cycle (reaction 1) and hepatic glutaminase (reaction 2) is decreased, whereas flux through hepatic glutamine synthetase (reaction 3) and renal glutaminase (reaction 4) is increased. This interorgan team effort between liver and kidney results in NH_4^+ disposal without concomitant HCO_3^- removal from the organism. From [116].

together with NH_4^+ during amino acid oxidation. In chemical terms, urea synthesis is an energy-driven neutralization of the strong base HCO_3^- by the weak acid NH_4^+:

$$2HCO_3^- + 2NH_4^+ \rightarrow \text{urea} + CO_2 + 3H_2O$$

Thus, a daily formation and excretion of 30 g of urea in man is equivalent to the disposal of 1 mol each of HCO_3^- and NH_4^+, i.e. amounts corresponding to the complete oxidation of about 100 g of protein [112]. This important function of hepatic ureogenesis received little attention in the past and its role was exclusively identified with the elimination of potentially toxic ammonia. Accordingly, increases or decreases of urea cycle flux relative to the rate of protein catabolism will diminish or expand the bicarbonate pool in the body respectively. In recent years it has become clear that urea cycle flux is sensitively controlled by the acid–base status. Several mechanisms have been identified which adjust NH_4^+ flux into urea according to the need to dispose of HCO_3^- in the sense of pH homeostasis (see below). In a well-balanced acid–base situation, the rate of bicarbonate removal (urea synthesis) from the organism must match the rate of bicarbonate production (protein catabolism); in line with this, urea cycle flux is normally adjusted to the rate of protein breakdown. However, in acidosis urea synthesis decreases relative to the rate of protein catabolism, resulting in a retention of HCO_3^- as a pH homeostatic response by the liver. Such a sparing of HCO_3^- by diminishing urea cycle flux in acidosis occurs without threat of hyperammonaemia, despite the ultimate link of HCO_3^- and NH_4^+ generation during protein catabolism. It is the structural and functional organization of nitrogen metabolizing pathways in the liver acinus which allows the uncoupling of urea synthesis from the need to dispose of ammonia and creates the basis for an independent control of ammonia and bicarbonate homeostasis [7,60,85,116]. The sequential organization of urea and glutamine synthesis in the liver acinus permits modulation of periportal urea cycle flux independent of the portal ammonia load, because perivenous scavenger cells act as an efficient back-up system for ammonia detoxication [85]; in acidosis, the liver switches ammonia detoxication from urea to net glutamine synthesis [62,76,85,111,119–121]. Renal glutamine hydrolysis and subsequent urinary NH_4^+ excretion (so-called renal ammoniagenesis) provide the final sink for surplus ammonia; both processes underly complex and sensitive short- and long-term regulation and are stimulated under acidotic conditions (for reviews see [110]). Thus, renal ammoniagenesis acts as a finely tuned spillover for the ammonia requiring elimination without concomitant HCO_3^- consumption and renal ammoniagenesis may represent a primary ammonia homeostatic response rather than a bicarbonate homeostatic one [7,60,62,68,111, 112,122]. Fig. 2 summarizes the co-ordinate interorgan team effort between liver and kidney for maintenance of HCO_3^- and NH_4^+ homeostasis. The concept depicted in Fig. 2 is at variance to traditional acid–base views in renal physiology and some aspects are still under controversial discussion [85,110,112–116, 122–126]. Today, this controversy no longer relates to experimental data or basic relationships between HCO_3^-, NH_4^+, protein breakdown and ureogenesis; it rather focusses on the question whether NH_4^+ is an important acid in a physiological context and how to define an acid in a living organism [124]. Clearly, at physiological pH values NH_4^+ is a very weak proton donor due to the $pK = 9.3$ of the NH_4^+/NH_3 system; NH_4Cl addition to or excretion from body fluids will have no relevant effect on pH, provided metabolism ceases [112–116,122]. On the other hand, however, it has been argued that because metabolic conversion of NH_4^+ to urea consumes HCO_3^-, NH_4^+ is an effective acid in the body and consequently NH_4^+ removal via urinary excretion is acid excretion [123–126]. These positions reflect the conceptual divergence between acid–base chemistry (Lowry–Bronstedt concept) and acid–base physiology starting in the third decade of this century (for review see [114]).

What are the mechanisms adjusting urea cycle flux to the needs of acid–base homeostasis? From our current knowledge, acid base control of urea synthesis is presumed to occur at the level of ammonia and bicarbonate delivery to carbamoyl phosphate synthetase, and possibly also at the level of carbamoyl phosphate synthetase activation by *N*-acetylglutamate [7]. Flux through liver glutaminase is very sensitive to small pH changes

[76–78,85–87,94]; in the presence of physiological glutamine and ammonia concentrations, lowering of the extracellular pH from 7.4 to 7.3 decreases glutaminase flux by about 70% [76,85]. This is due to a direct effect of pH on glutamine transporting systems, the activities of which determine the mitochondrial glutamine concentration [94], and a decreased ammonia [77,78] and hormone [76,127] activation of glutaminase. In addition, active uptake of amino acids into the liver is decreased in acidosis [94,100,128,129], which could contribute to glutaminase inactivation via cell volume regulatory mechanisms [91]. Although not observed in all species, a decrease of circulating plasma glutamine levels accompanying chronic metabolic acidosis in the rat [119,120,125] may not only lower substrate availability for hepatic glutaminase, but also serve as an important signal for a long-term down-regulation of liver glutaminase activity, both *in vitro* and *in vivo* [89]. Thus, acidosis switches off the mitochondrial ammonia amplifier glutaminase in periportal hepatocytes, and urea cycle flux decreases accordingly [85]. Another mechanism contributing to the inhibition of urea synthesis in acidosis could be a lowering of the free NH_3 concentration when the pH falls [130], because some evidence suggests that NH_3, not NH_4^+, is the reactive species at carbamoyl phosphate synthetase [131] and the activating species of glutaminase [78]. Whether an inhibition of *N*-acetylglutamate formation in acidosis as demonstrated *in vitro* [132] inhibits carbamoyl phosphate synthetase flux *in vivo* is not yet clear. A decreased hepatic amino acid oxidation in acidosis also tends to lower urea synthesis [128,129]. This, however, should have little impact on acid–base homeostasis, because inhibition of amino acid oxidation inhibits simultaneously the metabolic generation of bicarbonate; it is only the decrease in ureogenesis relative to the rate of protein and amino acid catabolism which results in a net gain of bicarbonate.

In addition to the glutaminase-controlled input of ammonia, the input of bicarbonate into carbamoyl phosphate synthetase reaction also is controlled by the acid–base status [38]. In view of the restricted permeability of the mitochondrial membrane to HCO_3^- (but not to CO_2) [133] and the HCO_3^- (not CO_2) requirement of carbamoyl phosphate synthetase [134], CO_2 conversion into HCO_3^- inside the mitochondria is required for urea synthesis. The spontaneous, uncatalysed hydratation reaction of CO_2 is too slow to maintain normal rates of urea synthesis. This explains the requirement of mitochondrial carbonic anhydrase V [36] for biosynthetic processes such as ureogenesis and gluconeogenesis [36–39,40,41]. Flux through mitochondrial carbonic anhydrase is very sensitive to small pH changes [36,38]; the enzyme is inhibited in acidosis and is an important site of urea cycle flux control [38,39,128]. On the other hand, the uncatalysed HCO_3^- provision for mitochondrial carbamoyl phosphate synthetase is pH-independent and increases with the extracellular CO_2 concentration. Thus, pH control of urea synthesis at the level of mitochondrial carbonic anhydrase is overridden at high CO_2 concentrations [38,39,135], and the CO_2 concentration determines the extent of pH control exerted by carbonic anhydrase on ureogenesis [38]. This explains why urea synthesis is less sensitive to pH changes in respiratory acidosis as compared to metabolic acidosis [38]. From a teleological point of view, such a differential response to respiratory and metabolic acidosis seems well designed for conditions when metabolic and respiratory compensation, respectively, have largely (but not completely) restored a physiological extracellular pH; i.e. only in (respiratorily compensated) metabolic acidosis, but not in (metabolically compensated) respiratory acidosis, a sparing of bicarbonate can correct the underlying acid–base derangement causally [38]. Interestingly, in contrast to ammonia delivered via the portal vein, glutamine-derived ammonia is incorporated into urea without control by mitochondrial carbonic anhydrase [39]. The mechanisms for this are not yet clear; possible explanations are discussed in [39]. However, because acid–base control of glutaminase is mediated by pH but not by the absolute HCO_3^- or CO_2 concentrations [86], this means that channelling of glutamine-derived ammonia into carbamoyl phosphate synthetase [80] proceeds unaffected by the acid–base status [7,39].

Urea synthesis is also controlled by the extracellular CO_2 and HCO_3^- concentrations even at a physiological extracellular pH. There is an absolute requirement for extracellular CO_2 (not CO_2 generated inside the mitochondria) for maintenance of urea cycle flux in perfused rat liver. In the absence of extracellular CO_2 and HCO_3^-, urea synthesis from NH_4Cl (but not from glutamine [38,39]) is completely inhibited in perfused rat liver [38,39,67] and increases with increasing extracellular CO_2 and HCO_3^- concentrations at constant pH [38]. This dependence is very steep at extracellular HCO_3^- concentrations below 10 mmol/l, but is also present in the range between 10 and 40 mmol/l [38]. Thus, even at a normal extracellular pH, *in vitro* the liver seems to respond to a decrease in buffer capacity of the extracellular HCO_3^-/CO_2 system with an inhibition of urea synthesis, and accordingly a sparing of bicarbonate.

In summary, the complex and sensitive control of ureogenesis by the three extracellular acid–base variables, i.e. pH, $[HCO_3^-]$ and $[CO_2]$ in experimental systems such as perfused rat liver seems well designed for an important contribution of the liver for maintenance of acid–base homeostasis *in vivo*; whenever the pH and/or the HCO_3^- concentration in the extracellular space fall, the liver responds with an inhibition of bicarbonate-consuming urea synthesis. It is the ultimate link between HCO_3^- and NH_4^+ formation during protein catabolism, which requires not only acid–base control of ureogenesis, but also mechanisms which adjust urea cycle flux to the rate of protein catabolism. These mechanisms, including roles for *N*-acetylglutamate and ornithine availability, have been reviewed in detail elsewhere [6,7]. Inhibition of urea synthesis in acidosis stimulates glutamine synthesis by increasing ammonia delivery to perivenous scavenger cells. This, together with a decreased periportal glutamine consumption, results in a net production of glutamine by the liver in acidosis [76,85] as demonstrated not only in the isolated perfused liver [76,89], but also *in vivo* [103,119–121].

PATHOPHYSIOLOGICAL ASPECTS

The discovery of the structural–functional organization of nitrogen metabolizing pathways in the liver acinus, including new regulatory sites for maintenance of both ammonia and bicarbonate homeostasis, has considerable pathophysiological impact. Although the concepts outlined above were established mainly in studies with the rat, several lines of evidence indicate that the situation may be similar in humans. These similarities not only include the sequential organization of urea [48] and glutamine synthesis [46,48] in the liver acinus, both pathways eliminating ammonia with low and high affinity respectively [68], and the kinetic properties of carbamoyl phosphate synthetase [136,137], but also ammonia activation of glutaminase [138], the occurrence of glutamine cycling [68] and a carbonic anhydrase requirement for urea synthesis [135]. Several pathophysiological aspects are listed below. The development of hyperammonaemia in liver disease has been ascribed to porto-systemic shunting and a loss of urea cycle enzyme activity [139,140]. There is now some evidence that a 4–5-fold compensatory increase of glutaminase activity ('mitochondrial ammonia amplifying system') in liver cirrhosis may represent a compensatory mechanism maintaining life-compatible urea cycle flux, despite a 80% reduction of urea cycle enzyme activities, and that pathogenesis of hyper-

ammonaemia involves a defect of the high-affinity system for ammonia detoxication in perivenous scavenger cells [68]. In this respect it is important to note that the capacity to synthesize glutamine is decreased by about 80% in liver cirrhosis [68]. Whether there is a disturbance of this scavenger function for compounds other than ammonia, such as biologically highly potent signal molecules, is at present unclear. However, a more general perivenous scavenging defect, as implicated by the perivenous scavenger cell hypothesis [70] could contribute to a variety of extrahepatic manifestations of chronic liver disease. Many acute liver diseases, such as poisoning with paracetamol, or viral hepatitis, are associated with the development of perivenous liver necrosis and hyperammonaemia may develop under these conditions due to a destruction of perivenous scavenger cells without impairment of periportal urea synthesis, as was shown in an experimentally induced perivenous necrosis following CCl_4 injury [25]. Thus, strategies aiming at the improvement of the perivenous scavenger cell function may be a promising therapeutic approach. Several diuretics behave as potent inhibitors of mitochondrial carbonic anhydrase V, thereby inhibiting hepatic urea synthesis already in therapeutic concentrations [135]. In healthy livers such an inhibition of periportal urea synthesis should be without effect on ammonium homeostasis, because of compensation by perivenous scavenger cells which guarantees an efficient ammonia detoxication back-up system by the liver. In liver cirrhosis, however, this scavenger is defective [68], and diuretic-induced inhibition of urea synthesis will give rise to hyperammonaemia. These phenomena explain simply why diuretics are well tolerated by patients with normal liver function, whereas they constitute in patients with liver cirrhosis a frequent precipitating cause of hyperammonaemia and hepatic encephalopathy, a phenomenon well known in clinical hepatology. The finding that glutamine synthetase could not be detected in human liver up to 2 days after birth [48], may represent a physiological condition of a perivenous scavenger cell defect. One is tempted to speculate if this contributes to the transient hyperammonaemia in premature new-borns [141,142]. Whether a reversal of sinusoidal blood flow in liver cirrhosis contributes to hyperammonaemia *in vivo* is not yet clarified. However, such circulatory changes should induce hyperammonaemia by reversing the sequence of the low- and high-affinity systems for ammonia detoxication, as was experimentally demonstrated in perfused rat liver [20].

A co-ordinate action of liver and kidney in maintenance of ammonia and bicarbonate homeostasis predicts that disturbances of either organ's function should lead to acid–base derangements. Indeed, chronic renal insufficiency and chronic liver insufficiency are frequently associated with metabolic acidosis and metabolic alkalosis, respectively [125,143,144]. Simple explanations for these acid–base disturbances are offered by the concepts outlined above (Fig. 2). In chronic renal failure, renal ammoniagenesis and urinary ammonia excretion are impaired [145,146]. Failure of this renal spillover function for NH_4^+ may lead to an inadequate ammonia stimulation of urea synthesis and consequently to an inadequately high bicarbonate consumption. Thus, when renal ammoniagenesis is impaired, hepatic urea synthesis prevents hyperammonaemia for the price of hypobicarbonataemia [116]. This situation resembles that following application of NH_4Cl to experimental animals, where metabolic acidosis develops from a stimulation of bicarbonate-consuming urea synthesis. On the other hand, impairment of hepatic urea synthesis in liver disease may diminish hepatic bicarbonate disposal and give rise to metabolic alkalosis [68,122]. This is supported by the demonstration that in man the *in vivo* plasma bicarbonate concentration increases with a progressive loss of urea cycle capacity (the latter determined *in vitro*), whereas no other causes for the development of a hyperbicarbonataemia (such as diuretic or antacid treatment, vomiting or hyperaldosteronism) were detectable [68,122]. Because alkalosis in turn is a potent stimulus of urea cycle flux by activating the ammonia amplifier glutaminase and increasing mitochondrial availability of HCO_3^- for carbamoyl phosphate synthetase, it seems that the development of a certain degree of hyperbicarbonataemia and alkalosis in liver cirrhosis produces a new, albeit more alkalotic, metabolic steady state maintaining life-compatible urea cycle flux, despite a reduction of urea cycle enzyme activities. Interestingly, the progressive loss of urea cycle capacity was paralleled by an increase of renal ammonia excretion, indicating that the kidney takes over the task of eliminating ammonia when urea synthesis fails [122].

PROSPECTS

Despite considerable advance in the understanding of hepatic function in controlling ammonia and bicarbonate homeostasis, many questions remain open. They relate to the factors controlling hepatocyte heterogeneity at the genome level, the intraacinar transfer of substrates and signals between different cell populations, a further metabolic characterization of perivenous scavenger cells and of inter-organ communications. In addition, parenchymal/non-parenchymal cell interactions and communications inside the liver acinus have gained interest in recent years (for review see [147]); their potential relevance for the present topic is yet far from being understood. It is hoped that this review article may help to stimulate further research in this area.

REFERENCES

1. Krebs, H. A. (1932) Klin. Wschr. **11**, 757–759
2. Krebs, H. A. (1935) Biochem. J. **29**, 1951–1959
3. Rappaport, A. M. (1976) Beitr. Pathol. **157**, 215–243
4. Lamers, W. H., Hilberts, A., Furt, E., Smith, J., Jonges, G. N., Van Noorden, C. J. F., Gaasbeek Janzen, J. W., Charles, R. & Moorman, A. F. M. (1989) Hepatology **10**, 72–76
5. Jungermann, K. & Katz, N. (1989) Physiol. Rev. **69**, 708–764
6. Meijer, A. J., Lamers, W. H. & Chamaleau, R. A. F. M. (1989) Physiol Rev., in the press
7. Häussinger, D., Meijer, A. J., Gerok, W. & Sies, H. (1988) in pH Homeostasis (Häussinger, D., ed.), pp. 337–377, Academic Press, London
8. Traber, P. G., Chianale, J. & Gumucio, J. J. (1988) Gastroenterology **95**, 30–43
9. Katz, N. (1989) Histochem. J. **21**, 517–529
10. Jungermann, K. & Katz, N. (1982) in Metabolic Compartmentation (Sies, H., ed.), pp. 411–435, Academic Press, London
11. Jungermann, K. (1987) Diabetes **3**, 269–293
12. Mizutani, A. (1968) J. Histochem. Cytochem. **16**, 172–180
13. Saheki, T., Yagi, Y., Sase, M., Nakano, K. & Sato, E. (1983) Biomed. Res. **4**, 235–238
14. Gebhardt, R. & Mecke, D. (1983) EMBO J. **2**, 567–572
15. Gaasbeek-Janzen, J. W., Lamers, W. H., Moorman, A. F. M., De Graaf, A., Los, A. J. & Charles, R. (1984) J. Histochem. Cytochem. **32**, 557–564
16. Lamers, W., Gaasbeek-Janzen, J. W., Moorman, A. F. M., Charles, R., Knecht, E., Martinez-Ramon, A., Hernandez-Yago, J. & Grisolia, S. (1988) J. Histochem. Cytochem. **36**, 41–47
17. Smith, D. D. & Campbell, J. W. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 160–164
18. Moorman, A. F. M., De Boer, A. J., Geerts, W. J. C., Zande, L. V. D., Lamers, W. H. & Charles, R. (1988) J. Histochem. Cytochem. **36**, 751–755
19. Gebhardt, R., Ebert, A. & Bauer, G. (1988) FEBS Lett. **241**, 89–93
20. Häussinger, D. (1983) Eur. J. Biochem. **133**, 269–274
21. Häussinger, D. & Gerok, W. (1983) Eur. J. Biochem. **136**, 421–425
22. Guder, W. G., Habicht, A., Kleißl, J., Schmidt, U. & Wieland, O. H. (1975) J. Clin. Chem. Clin. Biochem. **13**, 311–318
23. Stoll, B. & Häussinger, D. (1989) Eur. J. Biochem. **181**, 709–716

24. Häussinger, D., Stoll, B., Stehle, T. & Gerok, W. (1989) Eur. J. Biochem. **185**, 189–195
25. Häussinger, D. & Gerok, W. (1984) Chem. Biol. Interact. **48**, 191–194
26. Gebhardt, R., Burger, H. J., Schreiber, K. L. & Mecke, D. (1988) Hepatology **8**, 822–830
27. Kari, F. W., Yoshihara, H. & Thurman, R. G. (1987) Eur. J. Biochem. **163**, 1–7
28. Pösö, A. R., Penttilä, K. E., Suolinna, E. M. & Lindros, K. O. (1986) Biochem J. **239**, 263–267
29. Watford, M. & Smith, E. M. (1990) Biochem. J. **267**, 265–267
30. Burger, H. J., Gebhardt, R., Mayer, C. & Mecke, D. (1989) Hepatology **9**, 22–28
31. Quistorff, B. & Grunnet, N. (1987) Biochem. J. **243**, 87–95
32. Cooper, A. J., Nieves, E., Rosenspire, K. C., Filc-DeRicco, S., Gelbard, A. & Brusilow, S. W. (1988) J. Biol. Chem. **263**, 12268–12273
33. Taylor, P. M. & Rennie, M. J. (1987) FEBS Lett. **221**, 370–374
34. Carter, N., Jeffery, S., Legg, R., Wistrand, P. & Lönnerholm, G. (1987) Biochem. Soc. Trans. **15**, 667–668
35. Wolf, C. R., Moll, E., Freidberg, T., Oesch, F., Buchmann, A., Kuhlmann, W. D. & Kunz, H. W. (1984) Carcinogenesis **5**, 993–1001
36. Dodgson, S. J., Forster, R. E., Storey, B. T. & Mela, L. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 5562–5566
37. Dodgson, S. J., Forster, R. E., Schwed, D. A. & Storey, B. T. (1983) J. Biol. Chem. **258**, 7696–7701
38. Häussinger, D. & Gerok, W. (1985) Eur. J. Biochem. **152**, 381–386
39. Häussinger, D. (1986) Biol. Chem. Hoppe-Seyler **367**, 741–750
40. Metcalfe, H. K., Monson, J. P., Drew, P. J., Iles, R. A., Carter, N. & Cohen, R. D. (1985) Biochem. Soc. Trans. **13**, 255
41. Rognstad, R. (1983) Arch. Biochem. Biophys. **222**, 442–448
42. Gebhardt, R. (1988) Scand. J. Gastroenterol. **23** (Suppl. **151**), 8–18
43. Welsh, F. A. (1972) J. Histochem. Cytochem. **20**, 107–111
44. Shank, R. E., Morrison, G., Cheng, C. H., Karl, I. & Schwartz, R. (1959) J. Histochem. Cytochem. **7**, 237–239
45. Häussinger, D., Stehle, T. & Gerok, W. (1985) Hoppe-Seyler's Z. Physiol. Chem. **366**, 527–536
46. Gebhardt, R. & Mecke, D. (1984) in Glutamine Metabolism in Mammalian Tissues (Häussinger, D. & Sies, H., eds.), pp. 98–121, Springer Verlag, Heidelberg
47. Lamers, W. H., Hoynes, K. E., Zonnefeld, D., Moorman, A. F. M. & Charles, R. (1988) Anat. Embryol. **178**, 175–181
48. Moorman, A. F. M., Vermeulen, J. L. M., Charles, R. & Lamers, W. H. (1989) Hepatology **9**, 367–372
49. Campbell, J. (1990) in Comparative Animal Physiology (Prosser, C. H., ed.), Wiley, New York, in the press
50. Lamers, W. H., Gassbeek Janzen, J. W., te Kortschot, A., Charles, R. & Moorman, A. F. M. (1987) Differentiation **35**, 228–235
51. Gebhardt, R., Jirtle, R., Moorman, A. F. M., Lamers, W. H. & Michaelopoulos, G. (1989) Histochemistry **92**, 337–342
52. Gaasbeek Janzen, J. W., Moorman, A. F. M., Lamers, W. H. & Charles, R. (1985) J. Histochem. Cytochem. **33**, 1205–1210
53. Gaasbeek-Janzen, J. W., Gebhardt, R., Ten Voorde, G. H. J., Lamers, W. H., Charles, R. & Moorman, A. F. M. (1987) J. Histochem. Cytochem. **35**, 49–54
54. Gebhardt, R. & Mecke, D. (1979) Eur. J. Biochem. **100**, 519–525
55. Wong, B. S., Chenoweth, M. E. & Dunn, A. (1980) Endocrinology (Baltimore) **106**, 268–274
56. De Groot, C. J., Ten Voorde, G. H. J., Van Andel, R. E., Te Kortschot, A., Gaasbeek-Janzen, J. H., Wolson, R. H., Moorman, A. F. M., Charles, R. & Lamers, W. H. (1987) Biochim. Biophys. Acta **908**, 231–240
57. De Groot, C. J., Van Zonnevald, A. J., Mooren, P. G., Zonneveld, D., van den Dool, A., van den Bogaert, A. J. W., Lamers, W. H., Moorman, A. F. M. & Charles, R. (1984) Biochem. Biophys. Res. Commun. **124**, 882–888
58. De Groot, C. J., Zooneveld, D., de Laaf, R. T. M., Dingemanse, M. A., Mooren, P. G., Moorman, A. F. M., Lamers, W. H. & Charles, R. (1986) Biochim. Biophys. Acta **866**, 61–67
59. Gebhardt, R. & Mecke, D. (1979) Eur. J. Biochem. **97**, 29–35
60. Häussinger, D., Gerok, W. & Sies, H. (1984) J. Hepatol. **1**, 3–14
61. Cooper, A. J. L., Nieves, E., Coleman, A. E., Filc-DeRicco, S. & Gelbard, A. S. (1987) J. Biol. Chem. **262**, 1073–1080
62. Sies, H. & Häussinger, D. (1984) in Glutamine Metabolism in Mammalian Tissues (Häussinger, D. & Sies, H., eds.), pp. 78–97, Springer Verlag, Heidelberg
63. Lusty, C. (1978) Eur. J. Biochem. **85**, 373–383
64. Wanders, R. J. A., Van Roermund, C. W. T. & Meijer, A. J. (1984) Eur. J. Biochem. **142**, 247–254
65. Meijer, A. J., Lof, C., Ramos, I. & Verhoeven, A. J. (1985) Eur. J. Biochem. **148**, 189–196
66. Deuel, T. F., Louie, M. & Lerner, A. (1978) J. Biol. Chem. **253**, 6111–6118
67. Häussinger, D., Weiss, L. & Sies, H. (1975) Eur. J. Biochem. **52**, 421–431
68. Kaiser, S., Gerok, W. & Häussinger, D. (1988) Eur. J. Clin. Invest. **18**, 535–542
69. Häussinger, D., Stehle, T., Gerok, W., Tran-Thi, T.-A. & Decker, K. (1987) Eur. J. Biochem. **169**, 645–650
70. Häussinger, D. & Stehle, T. (1988) Eur. J. Biochem. **175**, 395–403
71. McGivan, J. D., Bradford, N. M., Verhoeven, A. J. & Meijer, A. J. (1984) in Glutamine Metabolism in Mammalian Tissues (Häussinger, D. & Sies, H., eds.), pp. 122–137, Springer Verlag, Heidelberg
72. McGivan, J. D. (1989) in Glutamine and Glutamate in Mammals (Kvamme, E., ed.), vol. 1, pp. 183–202, CRC Press, Boca Raton
73. Kovacevic, Z. & McGivan, J. D. (1983) Physiol. Rev. **63**, 547–605
74. Joseph, S. K. & McGivan, J. D. (1978) Biochim. Biophys. Acta **543**, 16–28
75. Häussinger, D. & Sies, H. (1979) Eur. J. Biochem. **101**, 179–184
76. Häussinger, D., Gerok, W. & Sies, H. (1983) Biochim. Biophys. Acta **755**, 272–278
77. Verhoeven, A. J., van Iwaarden, J. F., Joseph, S. K. & Meijer, A. J. (1983) Eur. J. Biochem. **133**, 241–244
78. McGivan, J. D. & Bradford, N. M. (1983) Biochim. Biophys. Acta **759**, 296–302
79. Meijer, A. J. & Verhoeven, A. J. (1986) Biochem. Soc. Trans. **14**, 1001–1004
80. Meijer, A. J. (1985) FEBS Lett. **191**, 249–251
81. Joseph, S. K., Verhoeven, A. J. & Meijer, A. J. (1981) Biochim. Biophys. Acta **677**, 506–511
82. Häussinger, D., Gerok, W. & Sies, H. (1984) Biochem. J. **236**, 261–265
83. Corvera, S. & Garcia-Sainz, J. A. (1983) Biochem. J. **210**, 957–960
84. Corvera, S. & Garcia-Sainz, J. A. (1982) Life Sci. **31**, 2493–2498
85. Häussinger, D., Gerok, W. & Sies, H. (1984) Trends Biochem. Sci. **9**, 300–302
86. Häussinger, D., Akerboom, T. M. & Sies, H. (1980) Hoppe-Seyler's Z. Physiol. Chem. **361**, 995–1001
87. Lueck, J. D. & Miller, L. L. (1970) J. Biol. Chem. **245**, 5491–5497
88. Rémésy, C., Morand, C., Demigné, C. & Fafournoux, P. (1988) J. Nutr. **118**, 569–578
89. McGivan, J. D. & Doyle, F. (1989) Abstr. Commun. FEBS Meet. 19th. no. TU 230
90. Lang, F., Stehle, T. & Häussinger, D. (1989) Pflueger's Arch. Physiol. **413**, 209–216
91. Häussinger, D., Lang, F., Bauer, K. & Gerok, W. (1990) Eur. J. Biochem. **188**, 689–695
92. McGivan, J. D., Lacey, J. H. & Joseph, S. K. (1980) Biochem J. **192**, 537–542
93. Häussinger, D., Soboll, S., Meijer, A. J., Gerok, W., Tager, J. M. & Sies, H. (1985) Eur. J. Biochem. **152**, 597–603
94. Lenzen, C., Soboll, S., Sies, H. & Häussinger, D. (1987) Eur. J. Biochem. **166**, 483–488
95. Kovacevic, Z. & McGivan, J. D. (1984) in: Glutamine Metabolism in Mammalian Tissues (Häussinger, D. & Sies, H., eds.), pp. 49–58, Springer Verlag, Heidelberg
96. Kilberg, M., Handlogten, M. E. & Christensen, H. N. (1980) J. Biol. Chem. **255**, 4011–4019
97. Fafournoux, P., Demigné, C., Rémésy, C. & LeCam, A. (1983) Biochem. J. **216**, 401–408
98. Joseph, S. K., Bradford, N. M. & McGivan, J. D. (1978) Biochem. J. **176**, 827–836
99. Jacob, R., Rosenthal, N. & Barrett, E. J. (1986) Am. J. Physiol. **251**, E509–E514
100. Häussinger, D., Lenzen, C. & Soboll, S. (1988) Contr. Nephrol. **63**, 161–166
101. Gebhardt, R. & Kleemann, E. (1987) Eur. J. Biochem. **166**, 339–344
102. Haynes, M. R. & McGivan, J. D. (1982) Biochem. J. **204**, 365–368
103. Welbourne, T. C. (1986) Biol. Chem. Hoppe-Seyler **367**, 301–305
104. DuRuisseau, J., Greenstein, J. P., Winitz, M. & Birnbaum, S. M. (1957) Arch. Biochem. Biophys. **68**, 161–171
105. Schimassek, H. & Gerok, W. (1965) Biochem. Z. **343**, 407–415
106. Lund, P. & Watford, M. (1976) in The Urea Cycle (Grisolia, S., Baguena, R. & Mayor, F., eds.), pp. 479–488, Wiley, New York

107. Addae, S. K. & Lotspeich, W. D. (1968) Am. J. Physiol. **215**, 278–281
108. Häussinger, D. & Gerok, W. (1984) Eur. J. Biochem. **143**, 491–497
109. Häussinger, D., Stehle, T. & Colombo, J. P. (1989) Biochem. J. **264**, 837–843
110. Häussinger, D. (ed.) (1988) pH Homeostasis, Academic Press, London
111. Oliver, J., Koelz, A. M., Costello, J. & Bourke, E. (1977) Eur. J. Clin. Invest. **7**, 445–449
112. Atkinson, D. E. & Camien, M. (1982) Curr. Top. Cell. Regul. **21**, 261–302
113. Atkinson, D. E. & Bourke, E. (1984) Trends Biochem. Sci. **9**, 297–300
114. Bourke, E. & Atkinson, D. E. (1988) in pH Homeostasis (Häussinger, D., ed.), pp. 163–179, Academic Press, London
115. Atkinson, D. E. & Bourke, E. (1987) Am. J. Physiol. **252**, F947–F956
116. Häussinger, D., Gerok, W. & Sies, H. (1986) Biochem J. **236**, 261–265
117. Bean, E. S. & Atkinson, D. E. (1984) J. Biol. Chem. **259**, 1552–1559
118. Randall, D. J., Wood, C. M., Perry, S. F., Bergman, H., Maloiy, G. M. O., Mommsen, T. P. & Wright, P. A. (1989) Nature (London) **337**, 165–166
119. Welbourne, T. C. (1988) in pH Homeostasis (Häussinger, D., ed.), pp. 379–401, Academic Press, London
120. Welbourne, T. C. (1987) Am. J. Physiol. **253**, F1069–F1076
121. Cersosimo, E., Williams, P. E., Radosevich, P. M., Hoxworth, B. T., Lacy, W. W. & Abumrad, N. N. (1986) Am. J. Physiol. **250**, E622–E628
122. Häussinger, D., Steeb, R. & Gerok, W. (1990) Klin. Wschr. **68**, 175–182
123. Walser, M. (1986) Am. J. Physiol. **250**, F181–F188
124. Alpern, R. J., Star, R. & Seldin, D. W.; reply by Atkinson, D. E. & Bourke, E. (1988) Am. J. Physiol. **255**, F807–F809
125. Brosnan, J. T., Vinay, P., Gougoux, A. & Halperin, M. L. (1988) in pH Homeostasis (Häussinger, D., ed.), pp. 281–304, Academic Press, London
126. Knepper, M. A. (1988) in pH Homeostasis (Häussinger, D., ed.), pp. 305–322, Academic Press, London
127. Kashiwagura, T., Erecinska, M. & Wilson, D. F. (1985) J. Biol. Chem. **260**, 407–414
128. Boon, L. & Meijer, A. J. (1988) Eur. J. Biochem. **172**, 465–469
129. Sestoft, L., Bartels, P. O. & Folke, M. (1982) Clin. Physiol. **2**, 51–58
130. Rémésy, C., Demigné, C. & Fafournoux, P. (1986) Eur. J. Biochem. **158**, 283–288
131. Cohen, N. S., Kyan, F. S., Kyan, S. S., Cheung, C. W. & Raijman, L. (1985) Biochem. J. **229**, 205–211
132. Kamamoto, E. S. & Atkinson, D. E. (1985) Arch. Biochem. Biophys. **243**, 100–107
133. Elder, J. A. & Lehninger, A. L. (1973) Biochemistry **12**, 976–982
134. Jones, M. E. (1976) in The Urea Cycle (Grisolia, S., Baguena, R. & Mayor, F., eds.), pp. 107–122, Wiley, New York
135. Häussinger, D., Kaiser, S., Stehle, T. & Gerok, W. (1986) Biochem. Pharmacol. **35**, 3317–3322
136. Pierson, D. L. & Brien, J. M. (1980) J. Biol. Chem. **255**, 7891–7895
137. Rubio, V., Ramponi, G. & Grisolia, S. (1981) Biochim. Biophys. Acta **659**, 150–160
138. Snodgrass, P. J. & Lund, P. (1984) Biochim. Biophys. Acta **798**, 21–27
139. Ugarte, G., Pino, M. E., Valenzuela, J. & Lorca, F. (1963) Gastroenterology **45**, 182–188
140. Kathra, B. S., Smith, R. B., Millikan, W., Sewell, C. W., Warren, W. D. & Rudman, D. (1974) J. Lab. Clin. Med. **84**, 708–715
141. Ballard, R. A., Vinocur, B., Reynolds, J. W., Wennberg, R. P., Merritt, A., Sweetman, L. & Nyhan, W. L. (1978) New Engl. J. Med. **299**, 920–925
142. Batshaw, M. L. & Brusilow, S. W. (1978) Pediatr. Res. **12**, 221–224
143. Oster, J. R. & Perez, G. O. (1986) J. Hepatol. **2**, 299–306
144. Dölle, W. (1965) Der Säurebasenstoffwechsel bei Leberzirrhose, Dr. A. Hüthig Verlag, Heidelberg
145. Welbourne, T. C., Weber, M. & Bank, N. (1972) J. Clin. Invest. **51**, 1852–1860
146. Tizianello, A., De Ferrari, G., Garibotto, G., Gurreri, G. & Robaudo, C. (1980) J. Clin. Invest. **65**, 1162–1173
147. Decker, K. (1985) Semin. Liver Dis. **5**, 170–195