REVIEW ARTICLE

The role of cellular hydration in the regulation of cell function*

Dieter HÄUSSINGER

Medizinische Universitätsklinik, Heinrich Heine Universität, Moorenstrasse 5, D-40225 Düsseldorf, Germany

INTRODUCTION

The cellular hydration state is dynamic and changes within minutes under the influence of aniso-osmolarity, hormones, nutrients and oxidative stress. This occurs despite the activity of potent mechanisms for cell volume regulation, which have been observed in virtually all cell types studied so far. These volume-regulatory mechanisms are apparently not designed to maintain absolute cell volume constancy; rather, they act as dampeners in order to prevent excessive cell volume deviations which would otherwise result from cumulative substrate uptake. On the other hand, these volume-regulatory mechanisms can even be activated in the resting state by hormones, and by this means changes in cell hydration are created. Most importantly, small fluctuations of cell hydration, i.e. of cell volume, act as a separate and potent signal for cellular metabolism and gene expression. Accordingly, a simple but elegant method is created for the adaptation of cell function to environmental challenges. In liver, cell swelling and shrinkage lead to certain opposite patterns of cellular metabolic function. Apparently, hormones and amino acids can trigger these patterns by altering cell volume. Thus cell volume homeostasis does not simply mean volume constancy, but rather the integration of events which allow cell hydration to play its physiological role as a regulator of cell function (for reviews see [1–4]). The interaction between cellular hydration and cell function has been most extensively studied in liver cells, but evidence is increasing that regulation of cell function through alterations of cell hydration also occurs in other cell types. This review will largely refer to hepatocytes, but when appropriate other cell types will also be considered. Regulation of mitochondrial function by hormone-induced changes of matrix volume has been established in the past (for reviews see [5,6]); this aspect will only be covered briefly. For further details, the reader is referred to recent surveys [2,4,7,8].

MECHANISMS OF CELL VOLUME CONTROL

Aniso-osmotic exposure of cells has been widely used to study cell volume regulation. Owing to the high water permeability of cell membranes and their inability to withstand significant hydrostatic pressure gradients, net water movements across the plasma membrane are driven almost exclusively by the osmotic gradient. In fact, when cells are suddenly exposed to hypo-osmotic media, they initially swell like more or less perfect osmometers, but within minutes they regain almost their original cell volume. This behaviour has been labelled regulatory cell volume decrease (RVD). Conversely, upon sudden exposure to hyperosmotic media, the cells shrink like osmometers, but display within minutes a regulatory volume increase (RVI), which brings back cell volume largely (but not completely) to the starting level. The mechanisms responsible for RVD and RVI may differ among different cell types and species, but in general involve the activation of ion transport systems in the plasma membrane. In addition, some cell types augment RVD and RVI by the release or accumulation of organic osmolytes.

Ionic mechanisms

The major cell-type-specific ionic mechanisms of cell volume regulation have been reviewed extensively in the past [2,3,7–13] and are schematically summarized in Figure 1. In rat liver, RVD is largely achieved by the release of cellular potassium, chloride and bicarbonate [9,14–19] following activation of Ba^{2+}- and quinidine-sensitive potassium channels in parallel with anion channels. It is unclear which intracellular signals activate these volume-regulatory responses in liver; however, tyrosine phosphorylation was recognized as an essential step in the RVD response in the human intestinal 407 cell line [20]. Hepatocyte swelling may open stretch-activated non-selective cation channels, which allow passage of Ca^{2+} into the cell [21]. The increase of intracellular Ca^{2+} then may activate Ca^{2+}-sensitive K^+ channels [22]. However, there is controversy as to whether hypo-osmotic hepatocyte swelling increases intracellular Ca^{2+} [23–25]. Thus Ca^{2+} activation of K^+ channels in liver may not be the only mechanism allowing RVD; indeed, K^+ channels have also been described in isolated hepatocytes which do not require an increase of intracellular Ca^{2+} [26]. It is conceivable that these K^+ channels are directly activated by cell membrane stretching, as is observed in rat proximal tubules [27]. A swelling-induced chloride conductance regulatory protein (termed pI_{Cln}) has been identified recently in Madin–Darby canine kidney (MDCK) and cardiac cells [28]. This protein is abundant in the cytosol and bound to cytosolic proteins such as actin, and may provide a link between cell swelling and opening of volume-regulatory Cl^- channels.

RVI in liver is achieved, at least in part, by parallel activation of Na^+/H^+ exchange and Cl^-/HCO_3^- exchange (Figure 1). Hyperosmotic exposure of perfused liver stimulates amiloride- and ouabain-sensitive K^+ uptake [17–19], which eventually leads to RVI. In contrast to other cell types, Na/K–2Cl co-transport appears not to participate appreciably in hepatic RVI, even though the carrier probably exists in the hepatic cell membrane and its activation by insulin is followed by an increase of cell volume [29,30].

Neither RVI nor RVD, however, completely restores the initial liver cell volume, i.e. the hepatocytes are left in either a slightly shrunken or a slightly swollen state. The extent of this volume deviation apparently acts as a signal which modifies cellular function.

Osmolytes

In addition to the ionic mechanisms of cell volume regulation, some cell types specifically accumulate or release organic compounds, so-called organic osmolytes, in response to cell shrinkage or cell swelling. Osmolytes need to be non-perturbing solutes that do not interfere with protein function even when occurring

Abbreviations used: RVD, regulatory volume decrease; RVI, regulatory volume increase; MDCK cells, Madin–Darby canine kidney cells; MAP kinase, mitogen-activated protein kinase; cAMP, cyclic AMP; PEPCK, phosphoenolpyruvate carboxykinase; SAP kinase, stress-activated kinase.

* Dedicated to Professor Dr. Dr. h.c. Wolfgang Gerok on the occasion of his 70th birthday.

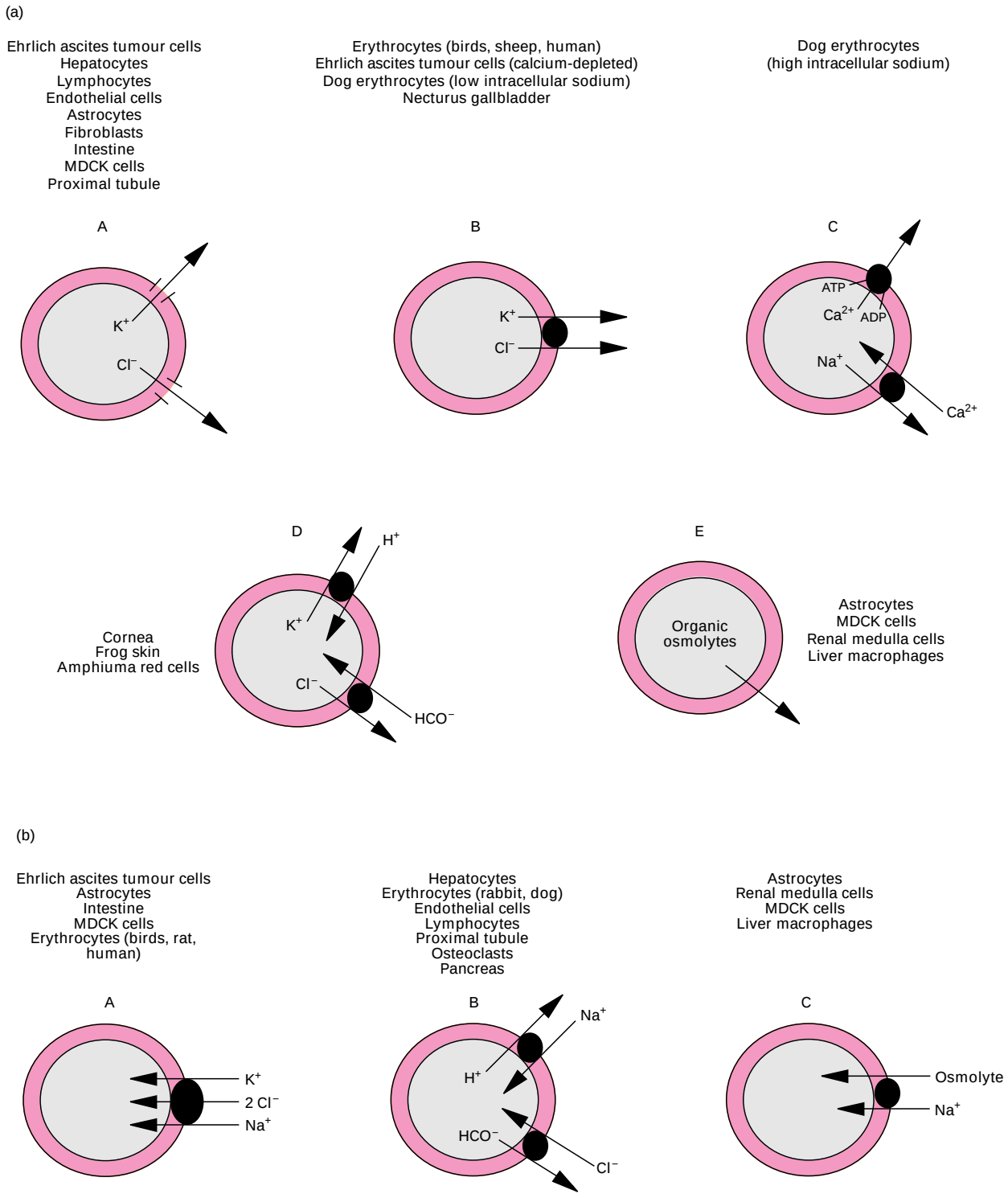

Figure 1 Major mechanisms of (a) RVD and (b) RVI following hypo-osmotic and hyperosmotic exposure respectively

The different ionic mechanisms of cell volume regulation are cell type- and species-dependent; in addition, some cell types use the accumulation or release of osmolytes. (**a**) Mechanisms of RVD: parallel activation of K^+ and Cl^- channels (A); K^+–Cl^- symport (B); parallel activation of Ca^{2+}-ATPase and Na^+/Ca^{2+} exchange (C); parallel activation of K^+/H^+ antiport and Cl^-/HCO_3^- exchange (D); release of organic osmolytes (E). (**b**) Mechanisms of RVI: activation of K^+/Na^+–$2Cl^-$ co-transport (A); parallel activation of Na^+/H^+ exchange and Cl^-/HCO_3^- antiport (B); cumulative Na^+-dependent uptake of organic osmolytes (C).

at high intracellular concentrations [4,7,31–33]. Such a prerequisite may explain why only a few classes of organic compounds, i.e. polyols such as inositol and sorbitol (e.g. in astrocytes, renal medulla and lens epithelial cells), methylamines such as betaine and α-glycerophosphocholine (e.g. in renal medulla) and certain amino acids such as taurine (e.g. in Ehrlich ascites

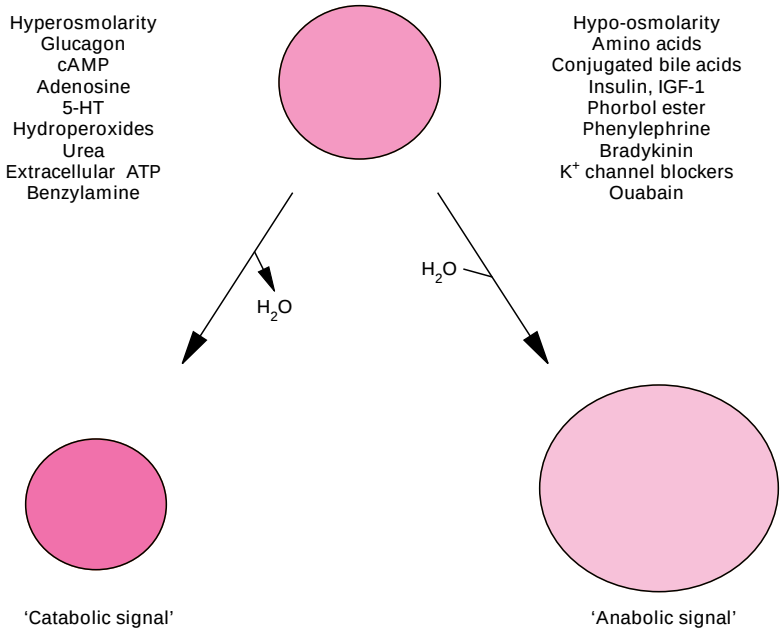

Figure 2 Modulators of liver cell hydration

Abbreviations: 5-HT, 5-hydroxytryptamine; IGF-1, insulin-like growth factor-1.

tumour cells) have evolved as osmolytes in living cells. Different mechanisms contribute to the intracellular accumulation of osmolytes during hyperosmotic stress: (i) decreased degradation (α-glycerophosphocholine), (ii) increased synthesis (induction of aldose reductase) and (iii) increased uptake following induction of specific Na^+-coupled transporters (e.g. for *myo*-inositol, betaine and taurine). Osmolytes in the renal medulla are especially important, since medullary fluid osmolarity can increase up to 3800 mosmol/l during antidiuresis and decrease to 170 mosmol/l during diuresis [34]. In the antidiuretic state (high extracellular osmolarity in the renal medulla), intracellular osmolarity increases in renal medullary cells as a result of the accumulation of inositol and betaine, which are taken up via Na^+-dependent transporters, and also as a result of increased synthesis of sorbitol and α-glycerophosphocholine. This process of intracellular osmolyte accumulation can produce intracellular organic osmolyte concentrations of several hundred mmol/l and intra/extracellular osmolyte concentration gradients of up to 1000 [32]. The enhanced synthesis of sorbitol from glucose by aldose reductase under these conditions involves an increased expression of the enzyme due to hyperosmotic activation of the encoding gene [35,36]. Likewise, the Na^+-dependent transporters for inositol and betaine are induced upon hyperosmotic exposure [33,36–39].

Organic osmolytes have not been identified with certainty in hepatocytes; however, betaine is an osmolyte in liver macrophages (Kupffer cells) and the expression of the betaine transporter is regulated by osmolarity in these cells (F. Zhang, U. Warskulat and D. Häussinger, unpublished work). In addition, a volume-activated taurine channel is present in skate hepatocytes [40], perfused rat liver releases small amounts of taurine in response to hypo-osmotic exposure [41], and rat hepatocytes possess a Na^+-coupled taurine transporter in the plasma membrane [42]. It is, however, unclear whether this taurine transporter in hepatocytes is osmoregulated (as is the taurine-transporting system β in mouse preimplantation conceptuses [43]) and whether taurine accumulation inside the hepatocytes is at all quantitatively relevant for cell volume regulation.

PHYSIOLOGICAL MODULATORS OF LIVER CELL VOLUME

Aniso-osmotic exposure may primarily be seen as an experimental tool to modify liver cell volume, although during intestinal absorption of water portal venous blood may become slightly hypotonic [44], and clinically relevant severe aniso-osmolarities (220–400 mosmol/l) due to hypo- and hyper-natraemia in plasma have been documented [45,46]. Physiologically more important, however, are cell volume changes due to cumulative substrate uptake [47–51], hormones and oxidative stress (Figure 2). Na^+-dependent amino acid transporters in the plasma membrane (for review see [52]) can build up intra/extracellular amino acid concentration gradients of up to 20-fold. Na^+ entering the hepatocyte together with the amino acid is extruded in exchange for K^+ by the electrogenic Na^+/K^+-ATPase. The accumulation of amino acids and K^+ in the cells leads to hepatocyte swelling, which in turn triggers volume-regulatory K^+ efflux [47,48,50,51] (Figure 3). This RVD, however, only prevents cell swelling from becoming excessive, and the hepatocytes remain in a swollen state as long the amino acid load continues. Cessation of amino acid infusion is followed by rapid cell shrinkage, and an RVI finally brings cell volume back to the starting level (Figure 3). Importantly, amino acid-induced cell swelling and volume-regulatory responses occur upon exposure to amino acids in the physiological concentration range [51], and physiological fluctuations in the portal amino acid concentration are accompanied by parallel alterations of liver cell volume. The degree of amino acid-induced cell swelling seems to be related largely to the steady-state intra/extracellular amino acid concentration gradient. This gradient, and accordingly the degree of cell swelling, is modified by hormones and the nutritional state in a complex way due to effects on the expression of plasma membrane transport systems, the electrochemical Na^+ gradient as a driving

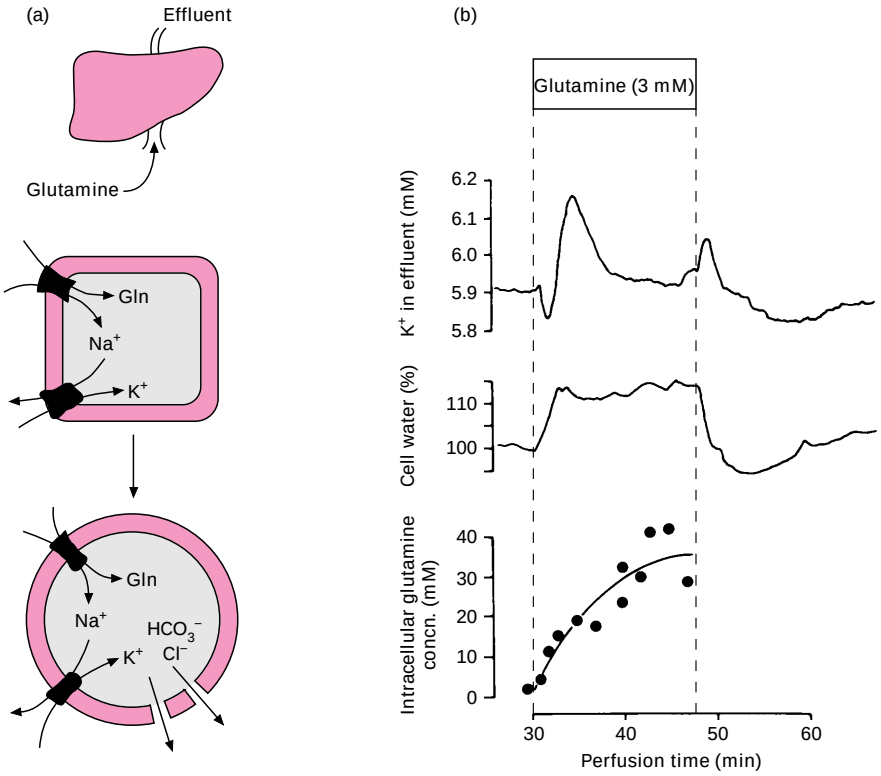

Figure 3 Effect of glutamine (3 mM) addition to the influent perfusate of isolated, single-pass perfused rat liver on intracellular glutamine accumulation, cell volume and volume-regulatory K^+ fluxes

(**a**) Glutamine leads to hepatocyte cell swelling due to cumulative, Na^+-dependent, uptake into liver cells and activates RVD due to K^+, Cl^- and HCO_3^- efflux. (**b**) Addition of glutamine to isolated perfused rat liver creates within about 12 min an intra/extracellular glutamine concentration gradient of about 12-fold. During the first 2 min of glutamine accumulation into the hepatocytes the liver cell volume increases rapidly, and hepatic net K^+ uptake during this phase is probably due to extrusion of Na^+ by the Na^+/K^+-ATPase. Thereafter, no further increase in cell volume is observed, despite the continuing accumulation of glutamine inside the cell. This is achieved by a volume-regulatory K^+ efflux from the liver which occurs until the steady-state intracellular glutamine concentration of about 35 mM is reached. The liver cell remains in a swollen state as long as glutamine is infused. The extent of cell swelling modifies cellular function. Reproduced from [48], with permission.

force for Na^+-coupled transport and alterations of intracellular amino acid metabolism. For example, in livers from fed rats, insulin, which is known to stimulate amino acid transport via system A, enhances glycine-induced cell swelling [29]. The concentrative uptake of conjugated bile acids also induces cell swelling [53].

Hormones are potent modulators of liver cell volume by affecting the activity of volume-regulatory ion transport systems [29,30,54–56]. In liver, insulin stimulates amiloride-sensitive Na^+/H^+ exchange [57], loop-diuretic-sensitive Na/K–2Cl co-transport [29,30] and the Na^+/K^+-ATPase [57,58], i.e. transport systems which are also turned on for RVI in liver and many other tissues. The concerted activation of these transporters leads to cellular accumulation of potassium, sodium and chloride, and consequently cell swelling. Insulin-induced cell swelling and cellular K^+ accumulation are abolished in the presence of bumetanide plus amiloride. Glucagon activates Na^+/K^+-ATPase [59], but simultaneously depletes cellular K^+ [30,54,55], probably due to a simultaneous opening of Ba^{2+}- and quinidine-sensitive K^+ channels [30]. As a result of the cellular Na^+, K^+ and probably Cl^- depletion, hepatocytes shrink (Figure 4). The physiological relevance is underlined by the finding that half-maximal effects of insulin and glucagon on liver cell hydration are found at hormone concentrations normally present in portal venous blood *in vivo*, i.e. 1.0 and 0.1 nM respectively [55]. Other hormones also modify hepatocellular hydration (Figure 2). Insulin-induced cell swelling is counteracted by glucagon. Insulin plus phenylephrine induces a 20 % increase in cell volume, whereas vasopressin plus glucagon leads to an approx. 20 % decrease. In order to achieve comparable changes of cell volume by aniso-osmotic exposure, extracellular osmolarity changes of about 100 mosmol/l are required [55].

Glucagon and Ca^{2+}-mobilizing hormones were also identified as modulators of mitochondrial matrix volume [5,6,60], but may affect the cytosolic and mitochondrial water spaces in opposite directions. For example, glucagon induces cell shrinkage and simultaneously swells the mitochondria. On the other hand, both cell and mitochondrial swelling occur under the influence of phenylephrine. For the mechanisms underlying the hormone-induced increase of mitochondrial matrix volume and the arising functional consequences, the reader is referred to refs. [5,6,60].

Oxidative stress exerted by hydroperoxides induces hepatocellular shrinkage due to the opening of Ba^{2+}-sensitive K^+ channels [61,62]. Cell shrinkage and K^+ channel opening also occur when hydrogen peroxide is generated intracellularly during the oxidation of monoamines. Evidence has been presented that the balance between intracellular metabolic H_2O_2 generation and its removal by detoxification systems such as catalase and glutathione peroxidase is one determinant for hepatocellular K^+ balance, and accordingly cell volume [62]. Oxidative stress may also lead to cell shrinkage in other cell types, as H_2O_2 stimulates

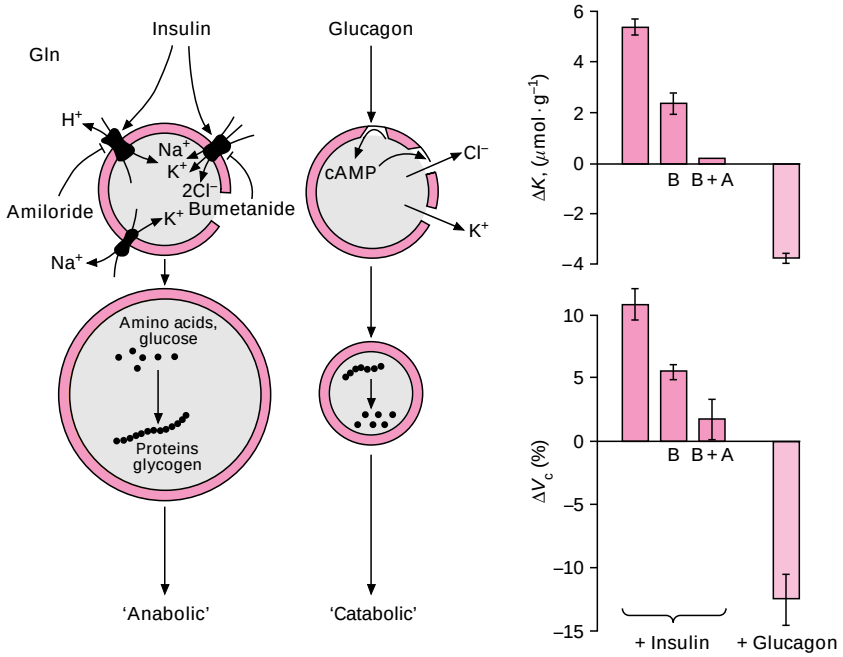

Figure 4 Modulation of liver cell volume and cellular K^+ balance by insulin and glucagon

Insulin activates amiloride-sensitive Na^+/H^+ exchange, bumetanide-sensitive Na/K–2Cl co-transport and the Na^+/K^+-ATPase, resulting in the cellular accumulation of K^+ (ΔK_i), Na^+ and Cl^- and cell swelling (ΔV_c). Both cellular K^+ accumulation and cell swelling induced by insulin are abolished in the presence of amiloride (A) plus bumetanide (B). Conversely, glucagon and cAMP deplete cellular K^+ by activating Cl^- channels and quinidine/Ba^{2+}-sensitive K^+ channels, thereby inducing cell shrinkage. The hormone-induced cell volume alterations act like another signal which participates in mediating the hormone effects on hepatic metabolism: cell swelling triggers an anabolic pattern and cell shrinkage a catabolic pattern of cellular function. Reproduced from [59a], with permission.

K^+ conductance in pancreatic B-cells [63] and oxidative stress inhibits Na/K–2Cl co-transport in vascular endothelial cells [64]. K^+ channel opening under the influence of urea at concentrations found in uraemia also induces cell shrinkage [65].

CELL VOLUME CHANGES AS A SIGNAL MODULATING CELL FUNCTION

Recent evidence suggests that the cellular hydration state is an important determinant of cell function and that hormones, oxidative stress and nutrients exert their effects on metabolism and gene expression in part by a modification of cell volume. The concept that cellular hydration acts as an independent signal on liver cell function is based on the following observations. (i) Persistent alterations of metabolism occur within minutes in response to aniso-osmotic cell volume changes (Table 1); these are fully reversible upon restoration of the resting cell volume. There is a dose–response relationship between the extent of the cell hydration change that remains after completion of volume-regulatory ion fluxes and the metabolic response. (ii) Intracellular signal transduction pathways are activated in response to changes in cell hydration. Their interruption by suitable inhibitors abolishes some anisotonicity-induced metabolic responses [66]. (iii) Several long-known, but mechanistically poorly understood, effects of amino acids which could not be related to their metabolism, such as stimulation of glycogen synthesis [67,68] or inhibition of proteolysis (for reviews see [69,70]), can be quantitatively mimicked by swelling the cells in hypo-osmotic media to the same extent as that induced by the amino acids [49,71–74]. This indicates that the metabolic effects exerted by these amino acids are due to their swelling potency. (iv) Several metabolic hormone effects can be mimicked by equipotent aniso-osmotic cell swelling or shrinkage, and some hormone effects disappear when the hormone-induced cell volume changes are prevented.

Thus cell hydration changes in response to physiological stimuli are an important and until recently unrecognized signal which helps to adapt cellular metabolism to alterations of the environment (substrate, tonicity, hormones) [1,73]. Consequently, Na^+-dependent amino acid transport systems in the plasma membrane should be viewed not merely as amino acid translocators; these transporters also act as transmembrane signalling systems by altering cellular hydration in response to substrate supply. Such a signalling role may shed new light on the long-known heterogeneity of transport systems among different cell types and their differential expression during development (for review see [52]), rendering specific amino acids as more or less potent signals for cell function. Likewise, transmembrane ion movements under the influence of hormones are an integral part of hormonal signal transduction mechanisms, with alterations of cellular hydration acting as another 'second messenger' of hormone action [1]. However, the exact place of hormone-induced cell volume changes in the network of known hormone-activated intracellular messenger systems remains to be established.

For example, insulin is capable of stimulating the mitogen-activated protein (MAP) kinase cascade through its own receptor tyrosine kinase. This initiates a series of other protein phosphorylations which bring about the effects of insulin on cell function. One of these may be activation of membrane ion transporters such as Na^+/H^+ exchange and Na/K–2Cl co-transport, resulting in cell swelling. Thus cell swelling in response to insulin may be a consequence rather than a cause of insulin

Table 1 Effects of hypo-osmotic cell swelling on liver function

Opposite functional patterns are triggered by hyperosmotic cell shrinkage. In general, liver cell swelling acts as an anabolic signal, whereas cell shrinkage is catabolic.

Liver cell swelling increases:
- Protein synthesis [84]
- Glycogen synthesis [56,71,90,91,95]
- Lactate uptake [19]
- Pentose phosphate shunt [74,92]
- Amino acid uptake [48,104]
- Glutamine breakdown [48]
- Glycine oxidation [74,85]
- Ketoisocaproate oxidation [85]
- Acetyl-CoA carboxylase [93,97]
- Lipogenesis [95]
- Urea synthesis from amino acids [88]
- MAP kinase activity [66]
- Glutathione (GSH) efflux [88]
- Taurocholate excretion into bile [24,53,74,107]
- Actin polymerization [124]
- Microtubule stability [76]
- Exocytosis [24,74,87,108,109]
- pH in vesicular compartments [25,82,112–114]
- mRNA levels of c-*jun* [128], ornithine decarboxylase [125], β-actin [124] and tubulin [76]

Liver cell swelling decreases:
- Proteolysis [29,49,54,72,73,76,77]
- Glycogenolysis [9,19,88]
- Glucose-6-phosphatase activity [170]
- Carnitine palmitoyltransferase I activity [96]
- Glutamine synthesis [48]
- Urea synthesis from NH_4^+ [88]
- Biliary GSSG release [92]
- Cytosolic pH [14,16,82,113]
- mRNA levels for PEPCK [127] and tyrosine aminotransferase*
- Viral replication [131]

* U. Warskulat and D. Häussinger, unpublished work.

action. On the other hand, hypo-osmotic cell swelling was shown to activate MAP kinases (see below) by itself, which may potentially result in a very complex interaction between cell volume and hormone action.

CELL-VOLUME-SENSITIVE METABOLIC PATHWAYS

Protein turnover

Hepatocellular hydration is a major point of proteolysis control in liver [73]: cell swelling inhibits, and conversely cell shrinkage stimulates, protein breakdown under conditions when the proteolytic pathway is not already fully activated [49,54,72,73]. There is a close relationship between proteolytic activity and hepatocellular hydration, regardless of whether the latter is modified by hormones, glutamine, glycine, alanine, bile acids, the K^+ channel blocker Ba^{2+} or anisotonic exposure (Figure 5), indicating that hydration changes are the common mechanism underlying proteolysis control by these heterogeneous effectors. The effects of glutamine, alanine, glycine, insulin, insulin-like growth factor-1 and glucagon on proteolysis can be mimicked quantitatively when the cell volume changes that occur in response to these effectors are induced to the same degree by aniso-osmotic exposure. Apparently the known anti-proteolytic effect of insulin and several (but not all) amino acids is transmitted in large part by agonist-induced cell swelling, whereas stimulation of proteolysis by glucagon is apparently mediated by cell shrinkage [29,49,54,73]. In line with this, the anti-proteolytic action of insulin disappears when insulin-induced cell swelling is prevented in the presence of inhibitors of the Na^+/H^+ antiporter and the Na^+/K^+–$2Cl^-$ co-transporter [73]. Furthermore, inhibition of proteolysis by glutamine and glycine is additive to the same extent as is observed with respect to cell swelling induced by both amino acids [49]. The nutritional state exerts its control on proteolysis by determining the swelling potencies of hormones and amino acids. For example, the anti-proteolytic effect of glycine in the fed state is only about one-third of that found after 24 h starvation, due to an approx. 3-fold higher swelling potency of glycine during starvation, which is explained by an up-regulation of the glycine-transporting amino acid transport system A [75]. Likewise, both the swelling potency and the anti-proteolytic effect of insulin are diminished in parallel by 60–70 % during starvation. It should be noted that not all amino acids exert their anti-proteolytic effect via changes of cell hydration; for example, leucine and phenylalanine are potent inhibitors of proteolysis, yet they exert little effect on cell volume. Here other mechanisms of proteolysis control apparently come into play.

The mechanisms by which cellular hydration exerts control on proteolysis are not clear; however, intact microtubular structures are required [76–78]. Disruption of microtubules by colchicine abolishes the anti-proteolytic action of hypo-osmotic, amino acid- or insulin-induced cell swelling [76,77], although colchicine itself has little effect on proteolysis. A requirement for intact microtubules may also explain why hypo-osmotic cell swelling was found to be without effect on proteolysis in freshly isolated rat hepatocytes [79]. However, the cell volume sensitivity of proteolysis reappears when cytoskeletal structures are reconstituted in culture [77]. The formation of autophagic vacuoles was shown to be controlled by protein phosphorylation/dephosphorylation [80,81], and current evidence suggests a role for protein phosphorylation in mediating the volume sensitivity of proteolysis. This could reside at the levels of ribosomal protein S6 phosphorylation [81], acidification of pre-lysosomal endocytotic/autophagic vesicular compartments [25,82] and possibly, but not yet proven, at the level of phosphorylation of microtubule-associated proteins.

Liver cell hydration also affects protein synthesis in the opposite direction to proteolysis: cell shrinkage inhibits, whereas cell swelling stimulates, protein synthesis [79,83,84]. The cyclic AMP (cAMP)-induced inhibition of protein synthesis in liver may, at least in part, be ascribed to cell shrinkage, because equipotent hyperosmotic cell shrinkage decreases protein synthesis in isolated rat hepatocytes to a comparable extent [84]. A close relationship exists between cell shrinkage and inhibition of protein synthesis under the influence of hyperosmotic exposure, cAMP and vasopressin [84]. On the other hand, insulin and phenylephrine have no significant effect on protein synthesis. However, when phenylephrine is added together with cAMP, the cAMP-induced inhibition of protein synthesis is potentiated although phenylephrine counteracts cAMP-induced cell shrinkage. As expected, cell volume changes may be only one factor among several mediating the hormonal control of protein synthesis.

Amino acid and ammonia metabolism

In rat liver, hypo-osmotic cell swelling switches the hepatic glutamine balance from net release to net uptake. This is due to a stimulation of flux through glutaminase in periportal hepatocytes and a simultaneous inhibition of glutamine synthesis in perivenous hepatocytes [48]. Opposite effects are found upon hyperosmotic exposure. Swelling-induced activation of glutaminase is most likely due to simultaneous mitochondrial swelling, which probably alters the attachment of the enzyme to

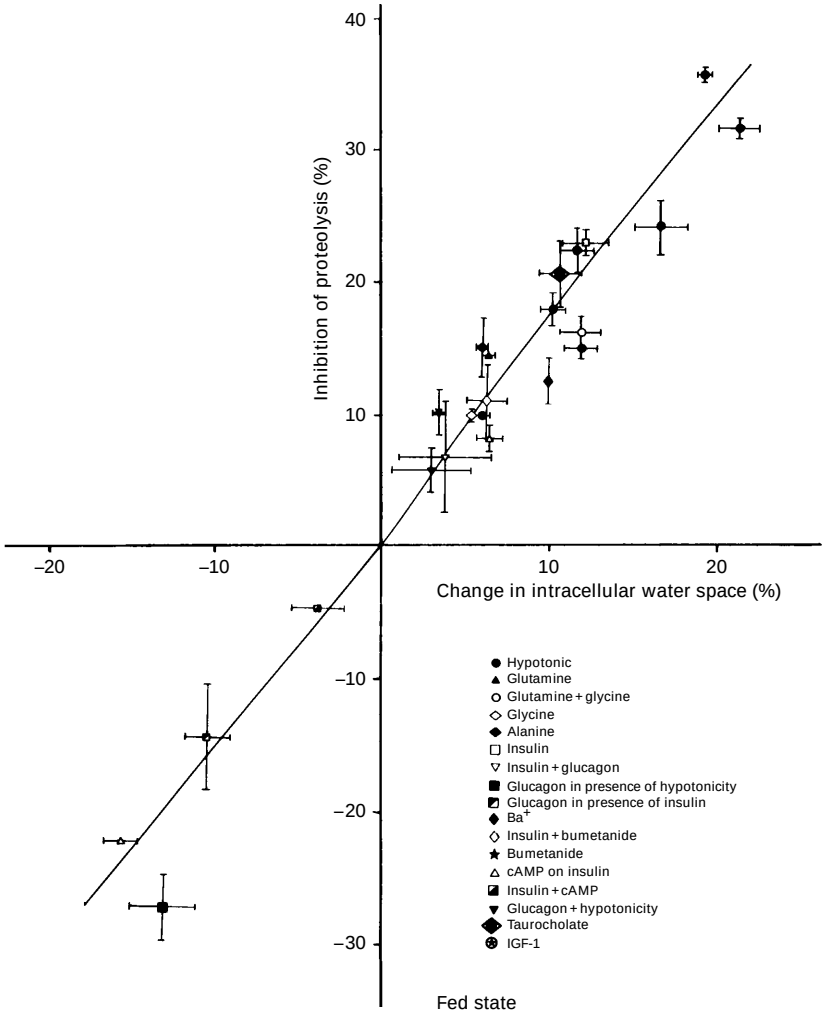

Figure 5 Relationship between cell volume and proteolysis in liver

Cell volume in perfused liver was determined as intracellular water space [112] and proteolysis was assessed as [^{3}H]leucine release in the effluent perfusate from perfused rat livers, which were prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine 16 h prior to the perfusion experiment. Cell shrinkage stimulates proteolysis, whereas cell swelling inhibits it. It should be noted that proteolysis is already maximally activated in the absence of hormones and amino acids and cannot be stimulated further by hyperosmotic or glucagon-induced cell shrinkage. The proteolysis-stimulating effect of these cell-shrinking manoeuvres, however, becomes apparent when proteolysis is pre-inhibited by either amino acids or insulin. Cell volume changes were induced by insulin, cAMP, glucagon, amino acids, Ba^{2+}, taurocholate or aniso-osmotic exposure. Abbreviation: IGF-1, insulin-like growth factor-1. Adapted from [73], with permission.

the mitochondrial inner membrane (for reviews see [5,6]). Likewise, hypo-osmotic swelling stimulates glycine oxidation in the perfused rat liver [85] and in isolated mitochondria [86]. These findings suggest that alterations of amino acid metabolism following aniso-osmotic cell volume changes can, at least in part, be explained by parallel alterations of mitochondrial matrix volume. Indeed, morphometric studies revealed transient mitochondrial swelling in isolated hepatocytes following hypo-osmotic exposure [87]. The effects of glucagon, cAMP and Ca^{2+}-mobilizing hormones on glutamine and glycine oxidation are also due to hormone-induced increases in mitochondrial matrix volume (for reviews see [5,6]), although these agents simultaneously induce liver cell shrinkage. Thus mitochondrial pathways, such as glutamine and glycine oxidation and respiration, are stimulated not only by hypo-osmolarity but also by cAMP, glucagon and Ca^{2+}-mobilizing hormones, despite opposing effects of these agents on cell volume.

In livers from fed rats, hypo-osmotic cell swelling stimulates urea synthesis and ammonia formation from amino acids [88], but inhibits these pathways when ammonia is used as the sole substrate for urea synthesis, although swelling of isolated mitochondria was shown to stimulate citrulline synthesis (for reviews see [5,6]). Inhibition of urea synthesis from ammonia by cell swelling is probably due to a block of the urea cycle at the step of argininosuccinate synthesis, as it is accompanied by an increase in citrulline but a decrease in aspartate, glutamate and malate tissue levels [88]. Simultaneously under these conditions the lactate/pyruvate ratio is increased whereas the β-hydroxybutyrate/acetoacetate ratio is decreased. These findings were interpreted to reflect a cell-swelling-induced disturbance of the transfer of reducing equivalents via the malate/aspartate shuttle and impaired aspartate regeneration during cell swelling. In line with this, the swelling-induced inhibition of urea synthesis from NH_4Cl was overcome by the addition of lactate and pyruvate. These substrates allowed intramitochondrial regeneration of oxaloacetate via pyruvate carboxylase, a reaction which was also shown to be stimulated by swelling of isolated mitochondria (for reviews see [5,6]).

Carbohydrate and fatty acid metabolism

Like protein turnover, carbohydrate metabolism in the liver is critically dependent upon the hepatocellular hydration state (Table 1). Hepatocyte swelling inhibits glycogenolysis, glycolysis [9,19,89] and glucose-6-phosphatase activity [90], but simultaneously stimulates glycogen synthesis [56,71,90,91], flux through the pentose phosphate pathway [92] and lipogenesis [93]. The opposite effects occur in response to cell shrinkage. It has become clear that the stimulatory effect of glutamine and other amino acids on glycogen synthesis and lipogenesis is due to amino acid-induced cell swelling [71]. Glycogen synthesis and lipogenesis are controlled by the activity of glycogen synthase and acetyl-CoA carboxylase; both of these enzymes are subject to regulation by phosphorylation/dephosphorylation. Swelling of isolated rat hepatocytes activates glycogen synthase in parallel with acetyl-CoA carboxylase [24,71,93,94] and decreases glycogen phosphorylase *a* activity, suggestive of interference by cellular hydration with protein phosphorylation. It was proposed that activation of glycogen synthase in response to hypo-osmotic cell swelling is, at least in part, due to a lowering of the intracellular chloride concentration, which relieves the inhibition of glycogen synthase phosphatase [90]. Activation of glycogen synthase by glutamate may contribute during glutamine-induced, but not during hypo-osmolarity-induced, cell swelling [90,93]. The swelling-induced stimulation of flux through the pentose phosphate shunt, and accordingly an increased NADPH provision for glutathione reductase, may explain why, during oxidative stress, the cellular losses of oxidized glutathione are smaller when the hepatocellular hydration state increases [92].

In the perfused rat liver, hypo-osmotic cell swelling stimulates, whereas hyperosmotic cell shrinkage inhibits, ketogenesis from ketoisocaproate, whereas ketogenesis from tyrosine, octanoate and glucose is largely uninfluenced [85]. However, with these substrates, hypo-osmotic (hyperosmotic) cell swelling (shrinkage) led to a decrease (increase) in the β-hydroxybutyrate/acetoacetate ratio [85]. The cell swelling-induced decrease in the β-hydroxybutyrate/acetoacetate ratio may be explained by a stimulation of the respiratory chain, as it occurs in response to mitochondrial swelling [5]. The inhibition of hepatic ketogenesis by glutamine, proline, alanine and asparagine is probably not due to amino acid-induced cell swelling, since no effect on ketogenesis was observed with the non-metabolizable amino acid analogue 2-aminoisobutyrate, despite cell swelling [95].

Hypo-osmotic incubation of hepatocytes slightly stimulates lipogenesis from glucose [95] and inhibits carnitine palmitoyltransferase [96]. Acetyl-CoA carboxylase, a key enzyme in fatty acid synthesis, is activated in response to hypo-osmotic- and amino acid-induced cell swelling [24]. As with glycogen synthase, activation of acetyl-CoA carboxylase is due to de-inhibition of a protein phosphatase, which occurs in response to a lowering of intracellular chloride and/or an increased intracellular concentration of glutamate and aspartate [97]. Lipogenesis is stimulated by glutamine, proline and alanine, but not by aminoisobutyrate, histidine or asparagine [95], and no correlation was detectable between the potency of these amino acids to swell the hepatocytes on the one hand and their potency to stimulate lipogenesis on the other [95]. This may suggest that volume changes *per se* may not play a major role in mediating the lipogenic effect of amino acids.

Plasma membrane transport

Not only are amino acids potent modulators of liver cell volume but, conversely, cell volume can exert control on amino acid transport. One obvious example is the induction of Na^+-dependent transport systems for neutral amino acids in a variety of cell types in response to hyperosmolarity ([98–101]; for review see [102]). Here, neutral amino acids apparently function as osmolytes in order to counteract cell shrinkage. However, amino acid transport may also be stimulated in response to cell swelling. In liver, the glutamine-transporting system N was shown to be activated by amino acids in a cycloheximide-insensitive way [103]. This stimulation was not explained by *trans*-stimulation and, in addition to substrates of system N, alanine, serine and the non-metabolizable analogue aminoisobutyrate, i.e. substrates of the Na^+-dependent system A, were also effective. This amino acid-dependent short-term stimulation of amino acid transport is due to cell swelling [104]. Hypo-osmotic cell swelling increases hepatic alanine and glutamine uptake [48], whose intracellular degradation rate was shown to be controlled by transport rather than by metabolism.

A swelling-induced hyperpolarization of the membrane [9] may not only augment Na^+-coupled substrate transport but may also explain why sinusoidal glutathione efflux from the liver is increased following hypo-osmotic liver cell swelling [88], since glutathione release was shown to be under the control of the membrane potential [105].

Bile excretion

In rat liver, conjugated bile acids are taken up across the canalicular membrane by a Na^+-dependent transporter, whereas canalicular excretion is thought to be predominantly accomplished by an ATP-dependent transport system (for reviews see [24,106]) and to represent the rate-controlling step for overall transcellular bile acid transport. In the perfused rat liver, the rate of transcellular taurocholate transport from the sinusoidal space into the biliary lumen is critically dependent upon the hydration state of the hepatocyte [53,107]. Cell shrinkage inhibits, whereas cell swelling stimulates, taurocholate excretion into bile, regardless of whether the cell volume is modified by anisotonic exposure or by amino acids [53]. The swelling-induced stimulation of taurocholate excretion into bile is due to an increase in transport capacity. An increase of hepatocellular hydration of about 10% leads within minutes to a doubling of the V_{max} of taurocholate excretion into bile [53]. This increase in V_{max} is not explained by changes in the cellular ATP content or the membrane potential, but is abolished in the presence of colchicine, indicating a requirement for intact microtubules for the interaction between cellular hydration and taurocholate excretion into bile [74]. From this, and from the substantial evidence for a transient stimulation of exocytosis as an early response to hypo-osmotic cell swelling [24,74,87,108,109], it was postulated that alterations in hepatocellular hydration induce rapid changes in the taurocholate secretion capacity due to a microtubule-dependent insertion/retrieval of canalicular bile acid transporter molecules into/from the canalicular membrane [24,53,74,108]. These transporters may be stored in an intracellular vesicular compartment underneath the canalicular membrane and could correspond to the known bile acid-containing vesicles, which were seen in the past to reflect 'vesicular transcellular bile acid transport'. However, the role of these vesicles may reside in the transport of the bile acid transporter molecules rather than in the transport of bile acids. Interestingly, the swelling-induced stimulation of taurocholate excretion is abolished in the presence of tyrosine kinase inhibitors or after pretreatment with cholera or pertussis toxin [110]. These inhibitors were also shown to block the swelling-induced activation of MAP kinases in rat hepatocytes [66,110], and this suggests a causal relationship between swelling-induced MAP kinase activation and the stimulation of

bile acid transport. It is not yet clear whether cell volume also affects other canalicular transport ATPases, such as the multi-specific organic anion transporter. However, recent studies on cysteinyl leukotriene excretion into bile in endotoxinaemia suggest this to be the case [111].

Acidification of endocytotic vesicles

In the liver, cell swelling (shrinkage) leads to a rapid alkalinization (acidification) of intracellular vesicular compartments, as revealed in studies on Acridine Orange fluorescence [112,113] and the fluorescence of endocytosed fluorescein isothiocyanate-labelled dextran [25,78,82]. The effects on vesicular pH occurred regardless of whether cell volume was modified by anisotonicity, insulin, amino acids, hydroperoxides or the K^+ channel blocker Ba^{2+} [82]. Subsequent studies revealed that the cell volume sensitivity of the vesicular pH reflects the response of an early endocytotic compartment (pH around 6), but not of late, more acidic, compartments (pH around 5), which are probably lysosomal [25]. Given the important role of vesicular acidification for receptor–ligand sorting, exocytosis and protein targeting [114,115], one is tempted to speculate that cellular hydration may also interfere with these processes. In addition, cell volume may interfere with receptor-mediated endocytosis: hyperosmotic exposure inhibits galactose-receptor-mediated endocytosis, but not fluid-phase endocytosis, in isolated hepatocytes [116]. Interestingly, pH control by the hydration state of endocytotic vesicles accessible to fluorescein isothiocyanate-labelled dextran is abolished after disruption of microtubules with colchicine [78,82], indicating again the involvement of the cytoskeleton.

The mechanism by which cell volume affects pH in endocytotic vesicles is not fully understood; however, it is mediated by a G-protein- and tyrosine-kinase-dependent, but Ca^{2+}- and cAMP-independent, mechanism [25]. This inhibitor sensitivity of pH regulation by cell volume in endocytotic vesicles again resembles that of the swelling-induced activation of MAP kinases [66]. Vesicular acidification requires the presence of a chloride conductance in the vesicular membrane in order to dissipate the membrane potential generated by the H^+ pump and to augment the acidification process [117]. Evidence suggests that chloride channel activity and acidification in endosomes prepared from calf brain or rabbit proximal tubule is regulated by protein kinase A-dependent phosphorylation [118,119], and phosphoproteins have been observed in endosomal membranes [120]. Although protein kinase A is not involved in the regulation of vesicular pH in liver [25], it is conceivable that other swelling-activated protein kinases (e.g. MAP kinases [25,66]) mediate the volume sensitivity of vesicular acidification by modulation of chloride channel activity [25].

CELLULAR HYDRATION AND GENE EXPRESSION

Cellular hydration affects cellular metabolism also on a long-term time scale by modifying gene expression. This involves not only osmoregulatory genes (whose mRNA levels increase in response to hypertonic stress), such as genes for aldose reductase or for osmolyte transporters such as the Na^+-coupled *myo*-inositol (SMIT) and betaine (BGT1) transporters in renal cells and astrocytes [32,37,121–123], but also the expression of genes coding for proteins which are not necessarily linked to osmoregulation. Examples of the latter are the hypo-osmolarity-induced increases in mRNA levels for β-actin [124], tubulin [76] and ornithine decarboxylase [125], the hyperosmolarity-induced stimulation of cyclo-oxygenase-2 expression in activated liver macrophages [126] and the cell volume-dependent expression in liver of phosphoenolpyruvate carboxykinase (PEPCK) [127], a key gluconeogenic enzyme. PEPCK mRNA levels markedly increase in response to hyperosmotic cell shrinkage but decrease in response to cell swelling both in the intact perfused rat liver and in cultured rat hepatoma H4IIE cells. Stimulation of proteolysis and induction of a key gluconeogenic enzyme in response to hypertonic cell shrinkage may be seen as an example of the joint regulation of functionally linked processes by cell volume. Aniso-osmotic exposure affects the expression of early immediate genes; examples are the increase in c-*jun* (but not c-*fos*) mRNA levels in response to liver cell swelling [128] and the induction of Egr-1 and c-fos mRNA following hyperosmotic treatment of MDCK cells [129]. In fibroblasts, amino acid deprivation leads to a cycloheximide-sensitive adaptive increase in the activity of amino acid transport system A [98]. This adaptive increase is potentiated by hyperosmotic cell shrinkage and counteracted by hypo-osmolar cell swelling, indicating again that cell volume modifies the expression of amino acid transport systems. Recent studies have drawn attention to amino acid-regulated gene expression in eukaryotic cells (for review see [130]). Although many signalling pathways may exist which link amino acid supply to gene expression, it is likely that amino acid-induced changes in cellular hydration contribute to this regulation.

Viral replication depends upon host cell hydration [131–134]. For example, hypo-osmotic swelling of duck hepatocytes inhibited replication of duck hepatitis B virus by about 50 %, whereas hyperosmotic shrinkage stimulated its replication 4–5-fold [131]. Surprisingly, cell shrinkage increased viral protein synthesis, whereas the synthesis of host cell proteins was decreased. In addition, evidence has been presented that viruses may interfere with ion transport across the plasma membrane [135–137], which could influence host cell hydration.

The mechanisms by which cell volume changes affect gene expression are largely unknown, but changes in ionic composition [35,138–140], the cytoskeleton [141] and protein phosphorylation [25,142] are likely candidates. Recent studies with MDCK cells have identified a hypertonic stress-responsive element in the 5′-flanking region of the mammalian BGT1 (betaine transporter) gene; however, the *trans*-acting factor(s) remain to be characterized [143]. A protein kinase C-dependent activation of MAP kinases in response to hypertonic stress has been described in MDCK cells [144]. However, the role of MAP kinases in inducing the betaine transporter is doubtful, as osmolyte transporter mRNA accumulation is still stimulated by hypertonicity in protein kinase C-depleted MDCK cells [145]. On the other hand, the swelling-induced induction of c-*jun* mRNA in hepatoma cells [128] may be due to MAP kinase activation [66]. Regulation of PEPCK mRNA levels by cellular hydration does not involve protein kinase C activation or changes in cAMP levels [127], but is sensitive to the protein kinase inhibitor H7 (U. Warskulat and D. Häussinger, unpublished work). The role of MAP kinases in the cell volume-dependent regulation of the PEPCK gene remains to be established. In yeast, evidence has been presented for a role of MAP kinase pathways in the regulation of the transcriptional activation of the glycerol synthetic pathway in response to high-salt conditions [146].

Alterations in gene expression, however, may also in turn affect cellular volume. This was suggested by an approx. 30 % increase in the resting-state cell volume following expression of the *ras* oncogene in NIH fibroblasts [147]. The growth factor-independent proliferation of cells expressing the *ras* oncogene is sensitive to amiloride and furosemide, i.e. to blockers of Na^+/H^+ antiport and Na/K–2Cl co-transport, suggesting a role for cell swelling induced by activation of these transporters in cell

proliferation. Furthermore, in lymphocytes, mitogenic signals activate these transporters and may shift the set-point of cell volume regulation to higher resting values [148]; this cell volume increase may be an important prerequisite for cellular proliferation.

Gene expression is affected not only by the cellular hydration state but also by the ambient colloid osmotic pressure. Evidence has been presented that the activity of a dominant liver transcription factor, hepatic nuclear factor-1α (HNF-1α), which controls the transcription of several liver-specific genes, is modulated by fluctuations in the level of oncotically active macromolecules [149].

SENSING OF THE CELLULAR HYDRATION STATE

In view of the multiple effects of cell hydration on cell function, the question arises as to how cell volume changes are sensed and how the signal is transduced to the level of cell function. Little is known yet about the structures that sense the changes in cell hydration. Because cell volume/hydration is a physical property of the cell, sensing should occur physically and/or mechanically. One hypothesis on physical volume sensing is that hydration changes will affect the concentrations of one or more intracellular constituents, which may act to influence volume-regulatory transport systems and/or intracellular signalling pathways. One intriguing model postulates that the extent of 'macromolecular crowding', i.e. the cytosolic protein concentration, will determine the tendency of intracellular macromolecules to associate with the plasma membrane and consequently their enzymic activity [150–152]. It is conceivable that cellular hydration may in such a way interfere with the activity of protein kinases and phosphatases, and changes in protein phosphorylation may trigger not only volume-regulatory responses [148,153] but also the volume-dependent alterations in cellular metabolism and gene expression.

Candidates for mechanical cell volume sensing are the cytoskeleton, recently identified ion-conductance regulator proteins [28] and so-called stretch-activated cation and anion channels, which have been identified in a variety of cell types and whose open-probability increases with membrane tension (for review see [154]). The molecular mechanisms of the stretch-activation of these channels are still unclear, although there is little doubt that such channels participate in cell volume regulation. Liberation of fatty acids from the membrane and interactions with the cytoskeleton have been discussed as initial events in the stretch-activation of ion channels. Recently, histidine kinases have been identified in yeast which are putative integral membrane proteins and may act as osmosensors [155], with the signal being transduced by autophosphorylation and subsequent phosphate transfer to an aspartate residue in the receiver domain of a cognate response regulator molecule in order to regulate a MAP-kinase-like protein kinase cascade.

INTRACELLULAR SIGNALLING EVENTS

A little more is known about the intracellular signalling events that couple changes in cell hydration to cell function, although the picture is incomplete and complicated by the fact that cell volume signalling may depend on the cell type under study. In addition, it is conceivable that the mechanism of how cell swelling is achieved (hypo-osmotic- versus amino acid-induced swelling) will influence cell volume signalling; for example, in jejunal enterocytes the RVD in response to cumulative substrate uptake was sensitive to inhibitors of protein kinase C, whereas the RVD following hypo-osmotic exposure was not [156]. Besides protein phosphorylation, the cytoskeleton and intracellular ions may be important for the link between cell hydration and cell function.

An important role for protein phosphorylation in cell volume regulation is suggested by the fact that volume-regulatory ion transporters such as the Na/K–Cl co-transporter and the Na^+/H^+ antiporter are regulated by phosphorylation. Okadaic acid, an inhibitor of protein phosphatases, increases cell volume in lymphocytes and hepatocytes, probably via phosphorylation-mediated activation of these transporters. Depending on the cell type under study, various protein kinases and phosphatases have been implicated in the mechanisms of cell volume regulation (for reviews see [2,3,148,153,155,157]); all of these could also participate in the regulation of cell function by cell volume. Current interest focuses on the regulation of MAP kinases and related protein kinases, such as Jnk [157], by osmotic stress. Hyperosmotic stress activates MAP kinases in yeast [147,157] and MDCK cells [144], whereas in rat hepatoma cells [66], rat hepatocytes [110,158], the human intestine 407 cell line [20] and primary astrocytes (F. Schliess, R. Sinning and D. Häussinger, unpublished work) MAP kinases are activated in response to hypo-osmotic cell swelling. A signal transduction sequence, which is initiated by the osmotic water shift across the plasma membrane and ultimately leads to changes in cell function, was recently identified in rat hepatoma and liver cells [66,110]. Here, hypo-osmotic cell swelling results within 1 min in a pertussis toxin-, cholera toxin- and genistein-sensitive, but protein kinase C- and Ca^{2+}-independent, phosphorylation of the MAP kinases Erk-1 and Erk-2 [66,110]. This suggests that liver cell swelling leads to a G-protein-mediated activation of an as-yet-unidentified tyrosine kinase, which acts to activate a pathway towards MAP kinases (Figure 6). The functional significance of this volume signalling pathway in liver is suggested by the findings that not only hypo-osmotic MAP kinase activation but also swelling-induced alkalinization and stimulation of bile acid excretion can be inhibited at upstream events, i.e. at the G-protein and tyrosine kinase level [25,65,110]. The finding that some metabolic responses to hypo-osmotic liver swelling are completely abolished by G-protein and tyrosine kinase inhibitors indicates not only that simple dilution of intracellular substrates following the osmotic water shifts cannot explain the cell volume-dependence of metabolism, but also that cell volume signalling may start at the plasma membrane. The intracellular signalling cascade which is initiated in response to liver cell swelling (Figure 6) resembles that triggered by growth factor receptor activation [159]. This similarity may explain why cell swelling acts like an anabolic signal in liver with respect to protein and carbohydrate metabolism (Table 1). MAP kinases have multiple protein substrates [159], such as the microtubule-associated proteins MAP-2 and Tau, other protein kinases, such as S6 kinase, and transcription factors such as c-Jun. In fact, the swelling-induced activation of MAP kinases is followed by increased phosphorylation of c-Jun, which may explain (due to autoregulation of the c-*jun* gene) the increase in c-*jun* mRNA levels 30 min after the onset of cell swelling [66,128]. However, besides Erk-1 and Erk-2, other Jun kinases may also be activated by cell swelling. A swelling-induced phosphorylation of transcription factors such as c-Jun may explain the influence of cell hydration on gene expression. A new subfamily of protein kinases, the stress-activated protein (SAP) kinases, has recently been described [160]. SAP kinases are activated by different forms of intra- and extra-cellular stress and act as c-Jun kinases; however, it remains to be established whether SAP kinases are also activated by osmotic stress. Protein phosphatases also participate in the regulation of cell function by cell hydration: hypo-osmotic hepatocyte swelling lowers the

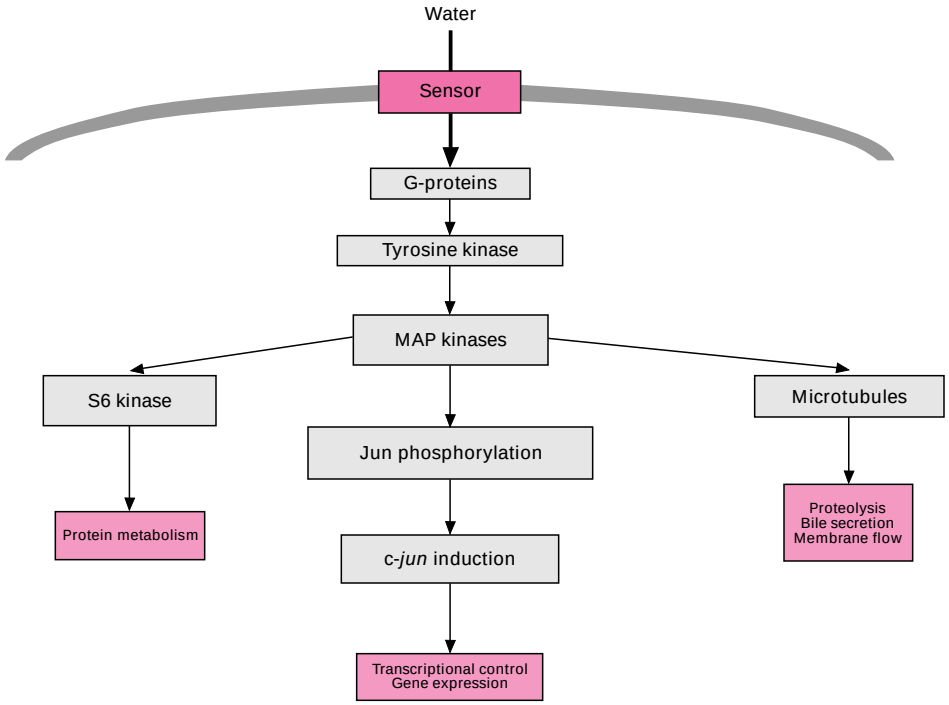

Figure 6 Cell volume signalling in the hepatocyte

The hypothetical scheme focuses on the role of MAP kinases, but it should be kept in mind that the signalling sequence depicted here is incomplete.

intracellular chloride concentration, thereby leading to a de-inhibition of glycogen synthase phosphatase [90].

Microtubules apparently play an important role in transducing some metabolic alterations in response to changes in cellular hydration. For example, disruption of microtubules by colchicine abolishes the swelling-induced alkalinization of endocytotic vesicles [82,78], inhibition of proteolysis [76–78] and stimulation of transcellular bile acid transport [74] in liver. It remains to be established to what extent changes in the phosphorylation of microtubule-associated proteins are involved in microtubule- and MAP-kinase-dependent cell volume signalling. On the other hand, other pathways which are activated in response to cell swelling, such as stimulation of glycine oxidation or of the pentose phosphate shunt, are not affected following microtubule disruption. Intact microtubules are not required for the swelling-induced activation of MAP kinases (F. Schliess and D. Häussinger, unpublished work). This suggests that microtubular structures are required during cell volume signalling at a step downstream of MAP kinases. Cell swelling leads within 1 min to an increased polymerization state of β-actin [124] and increases the stability of microtubules [76]; however, the relevance of actin filaments for cell volume signalling in liver is unknown. Microtubule stabilization found in *Ha-ras-1*-transformed Buffalo rat liver cells [162] may be explained by the increase in cellular hydration following *ras* oncogene expression [147].

Whereas in many cell types hypo-osmotic exposure is followed by an increase in intracellular Ca^{2+} (for review see [163]), this has not been observed consistently in hepatocytes [15,21,23,25,66]. Liver cell swelling decreases the intracellular pH [16,82,113], transiently hyperpolarizes the cell membrane potential [9] and stimulates inositol 1,4,5-trisphosphate formation [23,161]; however, translocation of protein kinase C to the membrane could not be detected (G. Finkenzeller and D. Häussinger, unpublished work). The significance of these alterations for the observed effects on hepatic metabolism remains to be established.

CLINICAL IMPLICATIONS

Many pathophysiological implications may arise from the interaction between cell hydration and cell function (for review see [164]); only a few can be addressed here. Contemporary clinical medicine pays careful attention to the hydration state of the extracellular space but not enough to cellular hydration, probably because of the lack of routinely applicable techniques for the assessment of cell volume in patients. The role of cellular hydration as an important determinant of protein turnover sheds new light on the understanding of protein-catabolic states in disease, and it has been hypothesized that cell shrinkage in skeletal muscle and liver may be the common end-path triggering protein catabolism in a variety of diseases [165]. Indeed, a close relationship between the cellular hydration state in skeletal muscle and the negativity of nitrogen balance was shown in severely ill patients, irrespective of the underlying disease [165].

The proposed dynamic system of bile acid carrier insertion/retrieval into/from the canalicular membrane in response to small changes in hepatocellular hydration may explain the beneficial effect of tauroursodeoxycholate in cholestasis [166]. In presence of taurocholate, tauroursodeoxycholate at low concentrations induces cell swelling, which leads to an increase in the V_{max} of taurocholate excretion into bile [53,74]. Hypo-osmotically swollen cells are apparently more resistant to hydroperoxide-induced cell damage [92,167], and in liver considerably higher concentrations of bile acids are required to induce the known cholestatic effect of taurocholate in swollen than in shrunken liver cells [53]. The role of cell hydration in organ preservation has not yet been elucidated.

Ammonia induces glial swelling as a result of glutamine accumulation in astrocytes. Both glial swelling and an increase in intracranial pressure were recognized as major events leading to brain dysfunction or death following acute ammonia intoxication or acute fulminant liver failure. In animal models of acute liver failure ammonia toxicity is reduced by inhibitors of glutamine synthetase [168], which also prevent glial swelling. Recent proton-NMR studies on the human brain *in vivo* indicate that astrocyte swelling is an early event in chronic hepatic encephalopathy, although an increase in intracranial pressure is clinically not detectable. Here a decrease in *myo*-inositol, a glial osmolyte, is found, indicative of glial swelling [46,169]. It was hypothesized that swelling-induced alterations in glia function may trigger disturbances of glial–neuronal communication, thereby provoking the clinical picture of chronic hepatic encephalopathy.

It is hoped that this review will stimulate further research in the area of cellular hydration, not only in basic but also in clinical science.

Our own work reported herein was supported by Deutsche Forschungsgemeinschaft, the Gottfried-Wilhelm-Leibniz-Prize, the Schilling-Stiftung and the Fonds der Chemischen Industrie.

REFERENCES

1 Häussinger, D. and Lang, F. (1992) Trends Pharmacol. Sci. **13**, 371–373
2 Lang, F. and Häussinger, D. (eds.) (1993) Interaction of Cell Volume and Cell Function, Springer Verlag, Heidelberg
3 Strange, K. (ed.) (1994) Cellular and Molecular Physiology of Cell Volume Regulation, CRC Press, Boca Raton
4 Häussinger, D., Gerok, W. and Lang, F. (1994) Am. J. Physiol. **267**, E343–E355
5 Halestrap, A. P. (1989) Biochim. Biophys. Acta **973**, 355–382
6 Halestrap, A. P. (1993) in Interactions of Cell Volume and Cell Function (Lang, F. and Häussinger, D., eds.), pp. 279–307, Springer Verlag, Heidelberg
7 Chamberlin, M. E. and Strange, K. (1989) Am. J. Physiol. **257**, C159–C173
8 Lang, F., Völkl, H. and Häussinger, D. (1990) Comp. Physiol. **4**, 1–25
9 Graf, J., Haddad, P., Häussinger, D. and Lang, F. (1988) Renal Physiol. Biochem. **11**, 202–220
10 Hoffmann, E. K. and Simonsen, L. K. (1989) Physiol. Rev. **69**, 315–382
11 McCarty, N. A. and O'Neil, R. G. (1992) Physiol. Rev. **72**, 1037–1061
12 Sarkadi, B. and Parker, J. C. (1991) Biochim. Biophys. Acta **1071**, 407–427
13 Fitz, G. (1994) Gastroenterology **107**, 1906–1907
14 Corasanti, J. G., Gleeson, D., Gautam, A. and Boyer, J. L. (1990) Renal. Physiol. Biochem. **13**, 164
15 Corasanti, J. G., Gleeson, D. and Boyer, J. L. (1990) Am. J. Physiol. **258**, G290–G298
16 Gleeson, D., Corasanti, J. G. and Boyer, J. L. (1990) Am. J. Physiol. **258**, G299–G307
17 Haddad, P., Thalhammer, T. and Graf, J. (1989) Am. J. Physiol. **256**, G563–G569
18 Häussinger, D., Stehle, T. and Lang, F. (1990) Hepatology **11**, 243–254
19 Lang, F., Stehle, T. and Häussinger, D. (1989) Pflügers Arch. **413**, 209–216
20 Tilly, B. C., van den Berghe, N., Tertoolen, L. G. J., Edixhoven, M. J. and de Jonge, H. R. (1993) J. Biol. Chem. **268**, 19919–19922
21 Bear, C. E. (1990) Am. J. Physiol. **258**, C421–C428
22 Bear, C. E. and Petersen, O. H. (1987) Pflügers Arch. **410**, 342–344
23 Baquet, A., Meijer, A. J. and Hue, L. (1991) FEBS Lett. **278**, 103–106
24 Boyer, J. L., Graf, J. and Meier, P. J. (1992) Annu. Rev. Physiol. **54**, 415–438
25 Schreiber, R. and Häussinger, D. (1995) Biochem. J. **309**, 19–24
26 Henderson, R. M., Graf, J. and Boyer, J. L. (1989) Am. J. Physiol. **256**, G1028–G1035
27 Sackin, H. (1987) Am. J. Physiol. **253**, F1253–F1262
28 Krapivinsky, G. B., Ackerman, M. J., Gordon, E. A., Krapivinsky, L. D. and Clapham, D. E. (1994) Cell **76**, 439–448
29 Hallbrucker, C., vom Dahl, S., Lang, F., Gerok, W. and Häussinger, D. (1991) Eur. J. Biochem. **199**, 467–474
30 Hallbrucker, C., vom Dahl, S., Lang, F., Gerok, W. and Häussinger, D. (1991) Pflügers Arch. **418**, 519–521
31 Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D. and Somero, G. N. (1982) Science **217**, 1214–1222
32 Garcia-Perez, A. and Burg, M. B. (1991) Physiol. Rev. **71**, 1081–1115
33 Burg, M. B. (1994) J. Exp. Zool. **268**, 171–175
34 Graf, J. (1993) in Interaction of Cell Volume and Cell Function (Lang, F. and Häussinger, D., eds.), pp. 67–117, Springer Verlag, Heidelberg
35 Uchida, S., Garcia-Perez, A., Murphy, H. and Burg, M. (1989) Am. J. Physiol. **256**, C614–C620
36 Ferraris, J. D., Williams, C. K., Martin, B. M., Burg, M. B. and Garcia-Perez, A. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 10742–10746
37 Kwon, H. M., Yamauchi, A., Uchida, S., Preston, A. S., Garcia-Perez, A., Burg, M. B. and Handler, J. S. (1992) J. Biol. Chem. **267**, 6297–6301
38 Takenaka, M., Preston, A. S., Kwon, H. M. and Handler, J. S. (1994) J. Biol. Chem. **269**, 29379–29381
39 Petronini, P. G., De Angelis, E., Borghetti, A. F. and Wheeler, K. (1994) Biochem. J. **300**, 45–50
40 Ballatori, N., Simmons, T. W. and Boyer, J. L. (1994) Am. J. Physiol. **267**, G285–G291
41 Brand, H. S., Meijer, A. J., Gustafson, L. A., Jorning, G. G., Leegwater, A. C., Maas, M. A. and Chamaleau, R. A. (1994) Biochem. Cell Biol. **72**, 8–11
42 Berkowitz, D., Hug, P., Sleight, R. G. and Bucuvalas, J. C. (1994) Am. J. Physiol. **267**, G932–G937
43 Van Winkle, L. J., Patel, M., Wasserlauf, H. G., Dickinson, H. R. and Campione, A. L. (1994) Biochim. Biophys. Acta **1191**, 244–255
44 Haberich, F. J., Aziz, O. and Nowacki, P. E. (1966) Pflügers Arch. **285**, 73–89
45 Lee, J. H., Arcinue, E. and Ross, B. D. (1994) N. Engl. J. Med. **331**, 439–442
46 Häussinger, D., Laubenberger, J., vom Dahl, S., Ernst, T., Bayer, S., Langer, M., Gerok, W. and Hennig, J. (1994) Gastroenterology **107**, 1475–1480
47 Bakker-Grunwald, T. (1983) Biochim. Biophys. Acta **731**, 239–242
48 Häussinger, D., Lang, F., Bauers, K. and Gerok, W. (1990) Eur. J. Biochem. **188**, 689–695
49 Hallbrucker, C., vom Dahl, S., Lang, F., Gerok, W. and Häussinger, D. (1991) Eur. J. Biochem. **197**, 717–724
50 Kristensen, L. O. and Folke, M. (1984) Biochem. J. **221**, 265–268
51 Wettstein, M., vom Dahl, S., Lang, F., Gerok, W. and Häussinger, D. (1990) Biol. Chem. Hoppe-Seyler **371**, 493–501
52 Kilberg, M. S. and Häussinger, D. (eds.) (1992) Mammalian Amino Acid Transport, Plenum Press, New York
53 Häussinger, D., Hallbrucker, C., Saha, N., Lang, F. and Gerok, W. (1992) Biochem. J. **288**, 681–689
54 vom Dahl, S., Hallbrucker, C., Lang, F., Gerok, W. and Häussinger, D. (1991) Biochem. J. **278**, 771–777
55 vom Dahl, S., Hallbrucker, C., Lang, F., Gerok, W. and Häussinger, D. (1991) Biochem. J. **280**, 105–109
56 Al-Habori, M., Peak, M., Thomas, T. H. and Agius, L. (1992) Biochem. J. **282**, 789–796
57 Jakubowski, J. and Jakob, A. (1990) Eur. J. Biochem. **193**, 541–549
58 Fehlmann, M. and Freychat, P. (1981) J. Biol. Chem. **256**, 7449–7453
59 Moule, S. K. and McGivan, J. D. (1990) Biochim. Biophys. Acta **1031**, 383–397
59a Häussinger, D. and Lang, F. (1991) Biochim. Biophys. Acta **1071**, 331–350
60 Halestrap, A. P., Davidson, A. M. and Potter, W. D. (1990) Biochim. Biophys. Acta **1018**, 278–281
61 Hallbrucker, C., Ritter, M., Lang, F., Gerok, W. and Häussinger, D. (1993) Eur. J. Biochem. **211**, 449–458
62 Saha, N., Schreiber, R., vom Dahl, S., Lang, F., Gerok, W. and Häussinger, D. (1993) Biochem. J. **296**, 701–707
63 Krippeit-Drews, P., Lang, F., Häussinger, D. and Drews, G. (1994) Pflügers Arch. **426**, 552–554
64 Elliott, S. J. and Schilling, W. P. (1992) Am. J. Physiol. **263**, H96–H102
65 Hallbrucker, C., vom Dahl, S., Ritter, M., Lang, F. and Häussinger, D. (1994) Pflügers Arch. **428**, 552–560
66 Schliess, F., Schreiber, R. and Häussinger, D. (1995) Biochem. J. **309**, 13–17
67 Katz, J., Golden, S. and Wals, P. A. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 3433–3437
68 Lavoinne, A., Baquet, A. and Hue, L. (1987) Biochem. J. **248**, 429–437
69 Mortimore, G. E. and Pösö, A. R. (1987) Annu. Rev. Nutr. **7**, 539–564
70 Seglen, P. O. and Gordon, P. B. (1984) J. Cell Biol. **99**, 435–444
71 Baquet, A., Hue, L., Meijer, A. J., van Woerkom, G. M. and Plomp, P. J. A. M. (1990) J. Biol. Chem. **265**, 955–959
72 Häussinger, D., Hallbrucker, C., vom Dahl, S., Lang, F. and Gerok, W. (1990) Biochem. J. **272**, 239–242
73 Häussinger, D., Hallbrucker, C., vom Dahl, S., Decker, S., Schweizer, U., Lang, F. and Gerok, W. (1991) FEBS Lett. **283**, 70–72
74 Häussinger, D., Saha, N., Hallbrucker, C., Lang, F. and Gerok, W. (1993) Biochem. J. **291**, 355–360
75 Hayes, M. R. and McGivan, J. D. (1982) Biochem. J. **204**, 365–368
76 Häussinger, D., Stoll, B., vom Dahl, S., Theodoropoulos, P. A., Markogiannakis, E., Gravanis, A., Lang, F. and Stournaras, C. (1994) Biochem. Cell Biol. **72**, 12–19

77 vom Dahl, S., Stoll, B., Gerok, W. and Häussinger, D. (1995) Biochem. J. **308**, 529–536
78 Busch, G. L., Schreiber, R., Dartsch, P. C., Völkl, H., vom Dahl, S., Häussinger, D. and Lang, F. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 9165–9169
79 Meijer, A. J., Gustafson, L. A., Luiker, J. J. F. P., et al. (1993) Eur. J. Biochem. **215**, 449–454
80 Holen, I., Gordon, P. B. and Seglen, P. O. (1993) Eur. J. Biochem. **215**, 113–122
81 Luiken, J. F. P., Blommaart, E. F. C., Boon, L., van Woerkom, G. M. and Meijer, A. J. (1994) Biochem. Soc. Trans. **22**, 458–461
82 Schreiber, R., Stoll, B., Lang, F. and Häussinger, D. (1994) Biochem. J. **303**, 113–120
83 Kruppa, J. and Clemens, M. J. (1984) EMBO J. **3**, 95–100
84 Stoll, B., Gerok, W., Lang, F. and Häussinger, D. (1992) Biochem. J. **287**, 217–222
85 Häussinger, D., Stoll, B., Morimoto, Y., Lang, F. and Gerok, W. (1992) Biol. Chem. Hoppe-Seyler **373**, 723–734
86 Brosnan, J. T., Jois, M. and Hall, B. (1990) in Amino Acids: Biochemistry, Biology and Medicine (Lubec, G. and Rosenthal, G. A., eds.), pp. 896–902, ESCOM Science Publishers BV, Leiden
87 Pfaller, W., Willinger, C., Stoll, B., Hallbrucker, C., Lang, F. and Häussinger, D. (1993) J. Cell. Physiol. **154**, 248–253
88 Häussinger, D., Lang, F., Bauers, K. and Gerok, W. (1990) Eur. J. Biochem. **193**, 891–898
89 Meijer, A. J., Baquet, A., Gustafson, L., van Woerkom, G. M. and Hue, L. (1992) J. Biol. Chem. **267**, 5823–5828
90 Grant, A., Tosh, D. and Burchell, A. (1992) Biochem. Soc. Trans. **21**, 39S
91 Peak, M., Al-Habori, M. and Agius, L. (1992) Biochem. J. **282**, 797–805
92 Saha, N., Stoll, B., Lang, F. and Häussinger, D. (1992) Eur. J. Biochem. **209**, 437–444
93 Baquet, A., Maisin, L. and Hue, L. (1991) Biochem. J. **278**, 887–890
94 Hue, L. (1994) Biochem. Soc. Trans. **22**, 505–508
95 Baquet, A., Lavoinne, A. and Hue, L. (1991) Biochem. J. **273**, 57–62
96 Guzman, M., Velasco, G., Castro, J. and Zammit, V. A. (1994) FEBS Lett. **344**, 239–241
97 Baquet, A., Gaussin, V., Bollen, M., Stalmans, W. and Hue, L. (1993) Eur. J. Biochem. **217**, 1083–1089
98 Gazzola, G. C., Dall'Asta, V., Nucci, F. A., Rossi, P. A., Bussolati, O., Hoffmann, E. K. and Guidotti, G. G. (1991) Cell. Physiol. Biochem. **1**, 131–142
99 Yamauchi, A., Miyai, A., Yokoyama, K., Itoh, T., Kamada, T., Ueda, N. and Fujiwara, Y. (1994) Am. J. Physiol. **267**, C1493–C1500
100 Soler, C., Felipe, A., Casado, J., McGivan, J. D. and Pastor-Anglada, M. (1993) Biochem. J. **289**, 653–658
101 Chen, J. G., Klus, L. R., Steenbergen, D. K. and Kempson, S. A. (1994) Am. J. Physiol. **267**, C529–C536
102 McGivan, J. D. and Pastor-Anglada, M. (1994) Biochem. J. **299**, 321–334
103 Weissbach, L. and Kilberg, M. S. (1984) J. Cell. Physiol. **121**, 133–138
104 Bode, B. and Kilberg, M. S. (1991) J. Biol. Chem. **266**, 7376–7381
105 Fernandez-Checa, J. C., Ren, C., Awe, T. Y., Ookhtens, M. and Kaplowitz, N. (1988) Am. J. Physiol. **255**, G403–G408
106 Nathanson, M. H. and Boyer, J. L. (1991) Hepatology **14**, 551–566
107 Hallbrucker, C., Lang, F., Gerok, W. and Häussinger, D. (1992) Biochem. J. **281**, 593–595
108 Bruck, R., Haddad, P., Graf, J. and Boyer, J. L. (1992) Am. J. Physiol. **262**, G806–G812
109 van Rossum, G. D. V., Russo, M. A. and Schisselbauer, J. C. (1987) Curr. Top. Membr. Transp. **30**, 45–74
110 Häussinger, D., Noe, B., Wettstein, M. and Schliess, F. (1995) in Cell Biology and Molecular Basis of Liver transport (Wehner, F. and Petringer, E., eds.), pp. 85–91, Project Verlag, Dortmund
111 Wettstein, M., Noe, B. and Häussinger, D. (1995) Hepatology, **22**, 235–240
112 Völkl, H., Friedrich, F., Häussinger, D. and Lang, F. (1993) Biochem. J. **295**, 11–14
113 Völkl, H., Busch, G. L., Häussinger, D. and Lang, F. (1994) FEBS Lett. **338**, 27–30
114 Dautry-Versat, A., Ciechanover, A. and Lodish, H. F. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 2258–2262
115 Tager, J. M., Aerts, J. M. F. G., Oude-Elferink, R. J. A., Groen, A. K., Hollemans, M. and Schram, A. W. (1988) in pH Homeostasis (Häussinger, D., ed.), pp. 123–162, Academic Press, London
116 Oka, J. A., Christensen, M. D. and Weigel, P. H. (1989) J. Biol. Chem. **264**, 12016–12024
117 van Dyke, R. (1993) Am. J. Physiol. **265**, C901–C917
118 Bae, H. R. and Verkman, A. S. (1990) Nature (London) **348**, 637–639
119 Rasenick, M. M., Wang, N. and Yan, K. (1990) in The Biology and Medicine of Signal Transduction (Nishizuka, Y., ed.), pp. 381–386, Raven Press, New York
120 Mulberg, A. E., Tulk, B. M. and Forgac, M. (1991) J. Biol. Chem. **266**, 20590–20593
121 Bedford, J. J., Bagnasco, S. M., Kador, P. F., Harris, W. F. and Burg, M. B. (1987) J. Biol. Chem. **262**, 14255–14259
122 Paredes, A., McManus, M., Kwon, H. M. and Strange, K. (1993) Am. J. Physiol. **263**, C1282–C1288
123 Yamauchi, A., Uchida, S., Preston, A. S., Kwon, H. M. and Handler, J. S. (1993) Am. J. Physiol. **264**, F20–F23
124 Theodoropoulos, T., Stournaras, C., Stoll, B., Markogiannakis, E., Lang, F., Gravani, A. and Häussinger, D. (1992) FEBS Lett. **311**, 241–245
125 Tohyama, Y., Kameji, T. and Hayashi, S. (1991) Eur. J. Biochem. **202**, 1327–1331
126 Zhang, F., Wettstein, M., Warskulat, U., Schreiber, R., Henninger, P., Decker, K. and Häussinger, D. (1995) Biochem. J. **312**, 135–143
127 Newsome, W. P., Warskulat, U., Noe, B., Wettstein, M., Stoll, B., Gerok, W. and Häussinger, D. (1994) Biochem. J. **304**, 555–560
128 Finkenzeller, G., Newsome, W. P., Lang, F. and Häussinger, D. (1994) FEBS Lett. **340**, 163–166
129 Cohen, D. M., Wasserman, J. C. and Gullans, S. R. (1991) Am. J. Physiol. **261**, C594–C601
130 Kilberg, M. S., Hutson, R. G. and Laine, R. O. (1994) FASEB J. **8**, 13–19
131 Offensperger, W. B., Offensperger, S., Stoll, B., Gerok, W. and Häussinger, D. (1994) Hepatology **20**, 1–7
132 Agol, V. I., Lipskaya, G. Y., Tolskaya, E. A., Voroshilova, M. K. and Romanova, L. I. (1970) Virology **41**, 533–540
133 Waite, M. R. F. and Pfefferkorn, E. R. (1968) J. Virol. **2**, 759–760
134 Bishop, J. M., Maldonado, R. L., Garry, R. F., Allen, P. T., Bosem H. R. and Waite, M. R. F. (1976) J. Virol. **17**, 446–452
135 Norrie, D. H., Wolstenholme, J., Howcroft, H. and Stephen, J. (1982) J. Gen. Virol. **62**, 127–136
136 Rey, O., Rossi, J. P. F. C., Lopez, R., Iapalucci-Espinoza, S. J. and Franze-Fernandez, M. T. (1988) J. Gen. Virol. **69**, 951–954
137 Pinto, L. H., Holsinger, L. J. and Lamb, R. A. (1992) Cell **69**, 517–528
138 Broeck, J. V., De Loof, A. and Callaerts, P. (1992) Int. J. Biochem. **24**, 1907–1916
139 Leake, R. E., Trench, M. E. and Barry, J. M. (1972) Exp. Cell Res. **71**, 17–26
140 Higgins, C. F., Cairney, J., Stirling, D. A., Sutherland, L. and Booth, I. R. (1987) Trends Biochem. Sci. **12**, 339–344
141 Hesketh, J. E. and Pryme, J. F. (1991) Biochem. J. **277**, 1–10
142 Santell, L., Rubin, R. L. and Levin, E. G. (1993) J. Biol. Chem. **268**, 21443–21447
143 Takenaka, M., Preston, A. S., Kwon, H. M. and Handler, J. S. (1994) J. Biol. Chem. **269**, 29379–29381
144 Itoh, T., Yamauchi, A., Miyai, A., Yokoyama, K., Kamada, T., Ueha,. N. and Fujiwara, Y. (1994) J. Clin. Invest. **93**, 2387–2392
145 Nakanishi, T., Balaban, R. S. and Burg, M. B. (1988) Am. J. Physiol. **255**, C181–C191
146 Brewster, J. L., de Valoir, T., Dwyer, D., Winter, E. and Gustin, M. C. (1993) Science **259**, 1760–1763
147 Lang, F., Ritter, M., Wöll, E., Weiss, E., Häussinger, D., Maly, K. and Grunicke, H. (1992) Pflügers Arch. Physiol. **420**, 424–427
148 Bianchini, L. and Grinstein, S. (1993) in Interaction of Cell Volume and Cell Function (Lang, F. and Häussinger, D., eds.), pp. 249–277, Springer Verlag, Heidelberg
149 Pietrangelo, A. and Shafritz, D. A. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 182–186
150 Minton, A. P., Colclasure, G. C. and Parker, J. C. (1992) Proc. Nat. Acad. Sci. U.S.A. **89**, 10504–10506
151 Parker, J. C. (1993) Am. J. Physiol. **265**, C1191–C1200
152 Colclasure, G. and Parker, J. C. (1991) J. Gen. Physiol. **98**, 881–887
153 Grinstein, S., Furuya, W. and Bianchini, L. (1992) News Physiol. Sci. **7**, 232–237
154 Sackin, H. (1994) in Cellular and Molecular Physiology of Cell Volume Regulation (Strange, K., ed.), pp. 215–240, CRC Press, Boca Raton
155 Maeda, T., Wurgler-Murphy, S. M. and Saito, H. (1994) Nature (London) **369**, 242–245
156 MacLeod, R. J., Lembessis, P. and Hamilton, J. R. (1992) Am. J. Physiol. **262**, C950–C957
157 Galcheva-Gargova, Z., Derijard, B., Wu, I. H. and Davis, R. J. (1994) Science **265**, 806–811
158 Agius, L., Peak, M., Beresford, G., Al Habori, M. and Thomas, T. (1994) Biochem. Soc. Trans. **22**, 516–522
159 Davis, R. J. (1993) J. Biol. Chem. **268**, 14553–14556
160 Kyriakis, J. M., Banerjee, P., Nikolakaki, E. et al. (1994) Nature (London) **369**, 156–160
161 vom Dahl, S., Hallbrucker, C., Lang, F. and Häussinger, D. (1991) Eur. J. Biochem. **198**, 73–83
162 Theodoropoulos, T., Gravanis, A., Saridakis, I. and Stournaras, C. (1992) Cell Biochem. Funct. **10**, 281–288

163 Foskett, K. J. (1994) in Cellular and Molecular Physiology of Cell Volume Regulation (Strange, K., ed.), pp. 259–277, CRC Press, Boca Raton
164 McManus, M. L. and Churchwell, K. B. (1994) in Cellular and Molecular Physiology of Cell Volume Regulation (Strange, K., ed.), pp. 63–77, CRC Press, Boca Raton
165 Häussinger, D., Roth, E., Lang, F. and Gerok, W. (1993) Lancet **341**, 1330–1332
166 Kitani, K. and Kanai, S. (1982) Life Sci. **30**, 515–523
167 Martins, E. A. and Meneghini, R. (1994) Biochem. J. **299**, 137–140
168 Hawkins, R. A., Jessy, J., Mans, A. M. and De Joseph, M. R. (1993) J. Neurochem. **60**, 1000–1006
169 Kreis, R., Ross, B. D., Farrow, N. A. and Ackerman, Z. (1992) Radiology **182**, 19–27
170 Grant, A., Tosh, D. and Burchell, A. (1992) Biochem. Soc. Trans. **21**, 39S