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PROSPECT

Carnitine: An Osmolyte That Plays a Metabolic Role

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Abstract Carnitine, gamma-trimethyl-beta-hydroxybutyrobetaine, is a small molecule widely present in all cells from prokaryotic to eukaryotic ones. It is the sole source of carbon and nitrogen in some bacteria; it serves as osmoprotectant in others. It is a carrier of acyl moieties, and exclusively of long-chain fatty acids for mitochondrial beta-oxidation in mammals. The conspicuously similar composition of the intracellular milieu among widely different species in relation to organic osmolyte systems involves the methylamine family to which carnitine belongs. This prompted us to examine the osmolytic properties of carnitine in an attempt to clarify the metabolic functions carnitine has acquired during evolution. An understanding of the metabolic functions of this organic compatible solute impinge on research involving this compound. J. Cell. Biochem. 80:1–10, 2000.† © 2000 Wiley-Liss, Inc.

Key words: osmolyte; carnitine; betaine; trimethylamine N-oxide; sarcosine; organic compatible solute

Carnitine, gamma-trimethyl-beta-hydroxybutyrobetaine, is a small molecule essential for long-chain fatty acid transfer into the mitochondrial matrix in which beta-oxidation occurs [Bremer, 1997]. Since carnitine is not metabolically destroyed by eukaryotic cells, carnitine homeostasis in mammals is regulated by: 1) a modest rate of endogenous synthesis; 2) absorption from dietary sources; and 3) efficient renal reabsorption [Rebouche et al., 1998]. Specific membrane carriers, able to modulate intracellular and extracellular carnitine pools, add to the complexity of this biological system. Although it is well established that carnitine is required for energy production by beta-oxidation, its concentration in various animal tissues does not consistently correlate with tissue energy requirements or lipid metabolism [Rebouche et al., 1998; Adams et al., 1998; Pessotto et al., 1997; Yamamoto and Turner, 1991].

As a member of the methylamine family, which includes betaine, trimethylamine N-oxide (TMAO) and sarcosine, carnitine can accumulate in osmotically stressed prokaryotic and eukaryotic cells, similarly to polyols and beta-amino acids [Poolman and Glaasker, 1998; Somero et al., 1992; Yamamoto and Turner, 1991; Wyn, 1984]. The function exerted by carnitine in osmoprotection in eukaryotes is not yet well defined, although it has been implicated in the stabilization of proteins and membranes, and in counteracting the effects of denaturating solutes [Scholte et al., 1996]. In this scenario, rather than being a lipid metabolism-related compound, carnitine should be viewed as an organic compatible solute. This shift in emphasis from a pure osmolyte to an osmolyte that exerts metabolic functions will have relevant implications in identifying new properties of carnitine in: 1) the maintenance of protein folding, aggregation of protein subunits, and stability of multiprotein complexes; 2) the maintenance of macromolecular function (enzymatic activity); 3) the counteraction of solute-induced denaturation of enzymes, i.e., urea; and 4) the correlation between osmotic changes and cell energy metabolic activities.

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Carnitine: An Organic Compatible Solute

Organic compatible solutes, small organic molecules (i.e., osmolytes), and inorganic ions constitute the bulk of the osmotically active solutes present in all cells. The fact that such phylogenetically diverse organisms as bacteria, unicellular algae, plants, invertebrates, and vertebrates use the same organic osmolytes suggests that there is a selective advantage associated with the use of these factors to modulate osmotic pressure [Yancey et al., 1982; Somero, 1986].

Cells accumulate energy-rich metabolites rather than more readily available inorganic ions such as K^+ and Na^+, in response to variations in osmotic pressure of the microenvironment. Indeed, although many biochemical functions require specific inorganic ions, increasing the concentrations of these ions often leads to disruption of enzyme activity. A high intracellular concentration of salts would seriously affect metabolic function as well as maintenance of proper transmembrane potentials [Yancey et al., 1982; Somero, 1986]. Osmolytes are specifically important for cell volume regulation in renal medulla, where extracellular osmolarity may become more than four-fold that of isotonicity, and in the brain, where cell volume alterations cannot be tolerated because of the rigid skull and where alterations of ion composition would affect excitability.

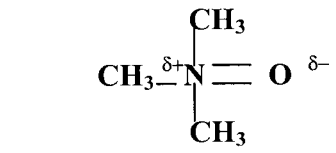
Organic osmolyte systems confer the selective advantage of compatibility with macromolecular structure and function at high or variable solute concentrations, hence there is less need for modifying proteins to function in concentrated intracellular solutions. There is evidence that osmolyte compatibility results from the absence of osmolyte interactions with substrates and cofactors, and the nonperturbing or favorable effects of osmolytes on macromolecular-solvent interactions [Yancey et al., 1982; Goldstein et al., 1996]. When cell solute content or extracellular osmolarity changes, the osmotic equilibrium is rapidly restored by transmembrane water flow which also causes cell swelling or shrinkage. Changes in cell volume activate specific metabolic and membrane pathways that result in the net accumulation or loss of osmotically active organic solutes, thus counteracting cell swelling or shrinkage [Strange et al., 1996; Kirk and Strange, 1998].

To circumvent the untoward effects of changes in ion composition, cells utilize so-called "osmolytes," molecules specifically designed to create osmolarity, without compromising other cell functions. Organic osmolytes are present at variable intracellular concentrations (i.e., tens to hundreds of millions per liter) in all organisms from bacteria to humans. They play a central role in cell osmoregulation, and may function as general cytoprotectants [Somero, 1986; McManus et al., 1995; Poolman et al., 1998]. These compatible solutes include three types of osmolytes: polyols (e.g., myo-inositol), methylamines (betaine, carnitine, TMAO, and sarcosine), and free amino acids (the beta-amino acids taurine and beta-alanine) (Fig. 1).

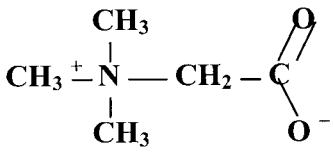
Clark and Zounes [1977] suggested that organic osmolytes are compatible with cell functions because of their structural similarities with the protein-stabilizing ions of the Hofmeister series, i.e., ions that tend to be excluded from the structured water around protein surfaces [Arakawa and Timasheff, 1985] (Fig. 2). For example, methylammonium ions, ammonium ion, sulfate, and acetate are protein stabilizers, and from their structure they resemble fragments of organic osmolytes.

In the Hofmeister series, the degree of methylation of the nitrogen atoms of substituted ammonium ions enhances their ability to stabilize macromolecules [Somero, 1986]. Thus, in the case of organic methylamines, it is not inconceivable that fully methylated compounds such as TMAO and carnitine, could be the most stabilizing organic osmolytes. Likewise, the ability of methylated glycine derivatives to offset the macromolecular destabilizing effects of salts depends on their degree of methylation [Yancey et al., 1982].

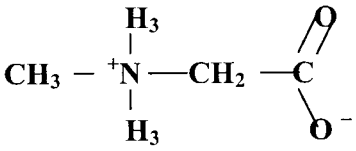
The reason why it was difficult to view carnitine as a compatible osmolyte is related to its positive charge. Indeed, most compatible organic osmolytes lack a positive charge that could form a complex with the generally negatively charged cell molecules. Polyols and TMAO are uncharged, and the other methylamines and amino acids are zwitterions that display a partial intramolecular charge neutralization in physiological pH range. Under physiological conditions, also carnitine exists as a zwitterion: the positive charge of the quaternary ammonium group is balanced by the



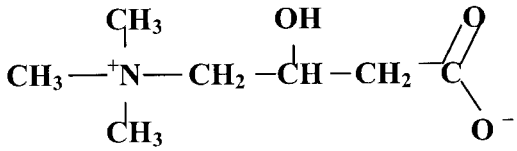
Trimethylamine N-Oxide (TMAO)



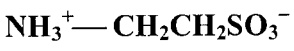
Betaine



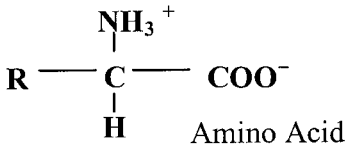
Sarcosine



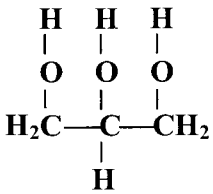
Carnitine



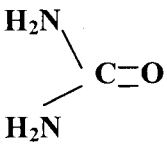
Taurine



Amino Acid



Glycerol



Urea

Fig. 1. Common intracellular organic osmolytes that exert stabilizing or perturbing effects on cell function.

negative charge of the ionized carboxylic acid group, which has a pK_a of 3.8 [Shaw et al., 1983; Zografi and Yalkowsky, 1970].

Studies on osmotic adaptation in prokaryotic cells led to the notion that carnitine can act as a compatible organic osmolyte. Indeed, carnitine, by uptake from the external medium under aerobic and anaerobic growth conditions [Verheul et al., 1998], allows Gram-negative bacteria such as *Escherichia coli* to grow at high osmolarity. Again, in Gram-positive bacteria such as *Listeria monocytogenes* and *Lactobacillus plantarum,* carnitine has been found to be a solute associated with osmotic adaptation [Beumer et al., 1994]. *Listeria monocytogenes* possesses highly specific separate transport systems for the osmolytes betaine and L-carnitine. Betaine transport proceeds via a proton-motive force-dependent carrier protein and L-carnitine is taken-up via an ATP-dependent transporter [Verheul et al., 1995]. In *L. plantarum*, betaine and L-carnitine enter

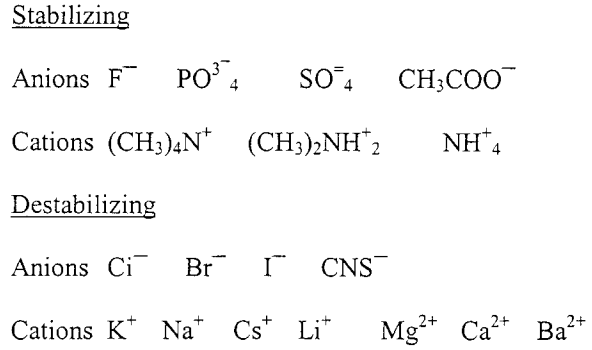


Fig. 2. Ions of the Hofmeister series.

the cytoplasm via the same transport system [Glaasker et al., 1996].

Alternative Role for Carnitine in Eukaryotic Cells: The Lens as a Paradigm

Carnitine first attracted attention because of its role in energy production by lipid beta-oxidation. Consequently, the presence of carnitine in tissues has invariably been investigated from a metabolic viewpoint. The canonical carnitine function fulfills two cell requirements: control of coenzyme A ester concentrations, which is rapid and not energetically expensive; and permeability of acyl moiety through membranes without the need to re-expend energy [Zammit, 1999].

Apart from metabolic functions, carnitine appears to exert other functions in mammals since the concentration of carnitine in diverse animal tissues is not always correlated with tissue energy requirements or lipid cell metabolism. The osmoprotectant function of carnitine in tissues in which the carnitine-dependent metabolic pathways do not coincide with the concentration of the molecule has not yet been investigated. A case in point is the lens [Pessotto et al., 1997]. The transparency of the crystalline lens depends on the regular or ordered spacing of its cells and proteins. Disturbance of this order, such as protein aggregation, membrane degeneration, fluctuations in protein density, and phase separation, causes local changes in the refractive index, which in turn induce light scattering [Bettelheim, 1985].

Studies of the spatial organization of lens fibers and lens proteins, and of the biological molecules involved in the maintenance of this organization might elucidate the mechanism of cataract formation [Cammarata et al., 1999]. Intracellular osmotic stress is linked to the progression of diabetic cataract. The lens compensates for water stress by accumulating osmotically active nonperturbing organic osmolytes [Garcia-Perez and Burg, 1991]. Three putative organic solutes have been identified in cultured lens epithelial cells, namely, sorbitol, myo-inositol, and taurine. As described above, the intracellular accumulation of these organic compatible osmolytes serves to maintain osmotic homeostates and to protect the cell against the perturbing effects of high intracellular concentrations of electrolytes that might otherwise adversely affect protein structure and function. But, while the lenticular epithelium can mobilize osmotic compensatory mechanisms, the fiber cells of the lens are characterized by increased susceptibility to osmotic damage largely because of an inability to adequately osmoregulate [Cammarata et al., 1997].

Studies from our laboratory showed that the decrease levels of carnitine in the eye in experimental diabetes is an early important and selective event [Pessotto et al., 1997]. In the eye, carnitine concentrations are highest in the lens [Pessotto et al., 1994], a non-vascularized tissue whose main source of energy is glucose absorbed from ocular fluids [Di Mattio and Zadunaisky, 1981].

As in other tissues, carnitine probably accumulates in lens against a concentration gradient by a mechanism of active transport. The decrease of lens carnitine in diabetes may be related to osmotic stress rather than to an intrisic pathological modification of the lens. This hypothesis is based on the premise that the main role of carnitine in the lens is not related to energy production but to the osmoprotectant role of the molecule. Being an organic compatible osmolyte, carnitine affects lens homeostasis by interfering with the biochemical modifications of lens proteins (Hofmeister effects) and by protecting the cells during physiological extracellular osmotic fluctuations. In cases of chronic aberrant osmotic pressure, as in diabetes, the net loss of carnitine irreversibly deranges fiber cell homeostasis and triggers the onset of cataract formation.

Physiological Effects Consequent to Carnitine Uptake and Release by Osmotic Pressure Regulation

In humans, it is reasonable to argue that carnitine has maintained the property of an

organic compatible osmolyte in all tissues affected by fluctuations in intracellular concentration related to the tonicity of the extracellular microenvironment. Experimental data on tissue carnitine uptake and efflux suggest that under physiological conditions, osmotic pressure-induced variations of carnitine within cells do not affect cell energy production, in other words, carnitine maintains its role in cell metabolic pathways. Tissue carnitine uptake is predominantly a Na^+-dependent, saturable process. The Na^+-dependent, high affinity human carnitine transporter has been identified [Tamai et al., 1998] and designated "OCTN2" because of its similarity (75.8%) to the organic cation transporter OCTN1, which is the low-affinity Na^+-dependent carnitine transporter. In adult tissues, OCTN2 is strongly expressed in kidney, skeletal muscle, placenta, heart, prostate, and thyroid, and weakly expressed in pancreas, liver, lung, brain, small intestine, uterus, thymus, adrenal gland, trachea, and spinal cord. In fetal tissues, OCTN2 is expressed at high levels in kidney and at very low levels in liver, lung, and brain. OCTN2-mediated carnitine transport is reduced by the structurally analogous compounds, D-carnitine, acetyl-D,L-carnitine, trimethyllysine, and gamma-butyrobetaine. Betaine less effectively reduces carnitine uptake, while gamma-aminobutyric acid, choline, beta-hydroxybutyrate, lysine and taurine are not inhibitory [Tamai et al., 1998; Stieger et al., 1995].

A separate system for carnitine efflux has been demonstrated in the liver; it is saturable, energy-independent, and inhibited by mersalyl but not by ouabain [Sandor et al., 1987]. A separate system for carnitine efflux out of cells has been identified in cultured heart cells and skeletal muscle [Molstad, 1980; Mesmer and Lo, 1990; Rebouche, 1977]. Moreover, an exchange carrier system has been demonstrated in heart tissue [Sartorelli et al., 1982, 1985], and skeletal muscle [Kerner and Hoppel, 1998]. In contrast to heart tissue and skeletal muscle, stimulation of carnitine efflux by extracellular carnitine was not demonstrated in hepatocytes, which suggests the liver lacks an exchange system [Sandor et al., 1985; Kispal et al., 1987].

Although cellular carnitine transport has been widely studied, there is yet no evidence that microenvironmental tonicity modulates membrane carnitine carriers. However, there are indications that in both physiological and pathological conditions in relation to osmotic pressure, cell carnitine increases or decreases. For example, in the early stages of lactation, the rat and ovine mammary gland, generates a substantial transepithelial carnitine gradient in favor of milk [Shennan et al., 1998]. The substrate specificity of carnitine uptake by mammary tissue taken from lactating rats is characterized by a saturable Na^+-dependent component and a nonsaturable Na^+-independent component, the former is inhibited by D-carnitine and acetyl-L-carnitine, but not by choline or taurine. Carnitine efflux is characterized by a fast and slow component, it is not greatly affected by Na^+, and is neither stimulated nor inhibited by increases in external carnitine concentration [Shennan et al., 1998]. There is a strong possibility that volume-sensitive L-carnitine efflux uses the volume-sensitive organic osmolyte/anion channel (VSOAC) [Shennan et al., 1998].

Interestingly, the VSOAC, which accepts organic compounds as substrates, has a broad substrate specificity. Normally, a hypoosmotic shock, hence cell swelling, stimulates the efflux of low molecular weight compounds (i.e., taurine and glycine) from mammary tissue explants. Upon hypoosmotic challenge, the fractional efflux of carnitine increases by about 40% [Shennan et al., 1998].

At this point, one may speculate that the efflux of carnitine, regulated by the osmolarity of the microenvironment, is an important physiological mechanism that serves to enhance the nutritional value of milk. In this case, rather than controlling the extracellular compartment tonicity, the efflux of carnitine from cells serves to supply carnitine to the neonate via milk. Indeed, the capacity of the newborn to synthetize carnitine is very low during the early stages of lactation, while the carnitine-dependent oxidation of long-chain fatty acids is particularly important for the provision of acetyl units required for the synthesis of complex lipids in the developing brain. In this context, the osmoregulatory pathways are exploited to increase the carnitine concentration in milk, although this regulation depletes the mother's hepatic reserve of carnitine [Shennan et al., 1998].

The osmoregulatory properties of carnitine are also crucial to the kidney, where an extra-

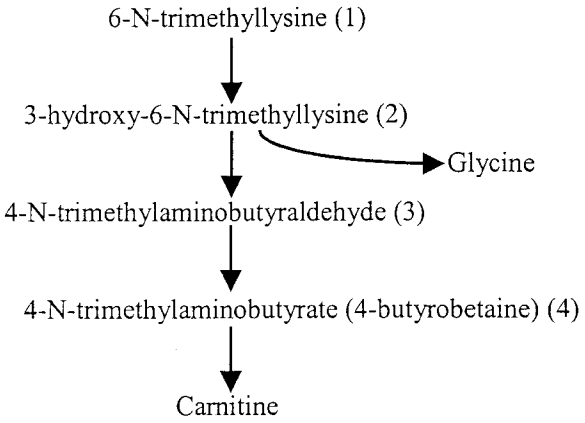


Fig. 3. Carnitine synthesis. (1) 6-N-trimethyllysine hydroxylase; (2) 3-hydroxy-6-N-trimethyllysine aldolase; (3) aldehyde dehydrogenase; (4) 4-N-butyrobetaine hydroxylase.

cellular hypertonicity is normally present. Indeed, while in mammalian tissue most cells are exposed to extracellular fluid with well-controlled osmolarity, a notable exception is the kidney medulla, where extracellular osmolarity can approach values exceeding isotonicity by a factor of >4 [Lang et al., 1998]. Medullary cells have to withstand this excessive extracellular osmolarity for prolonged periods. However, they also undergo rapid changes of osmolarity during transition from antidiuresis to diuresis, when medullary osmolarity rapidly decreases toward isoosmolarity. In renal tissue, five organic osmolytes actively accumulate intracellularly in the medulla to balance high external solute concentrations and minimize the osmolyte gradient across the cell membrane [Miyai et al., 1996; Burg et al., 1997]. These molecules are betaine, sorbitol, inositol, glycerophosphorylcholine, and taurine.

As mentioned above, methylamines, such as betaine, are also counteracting solutes that offset the protein destabilizing effects of urea without disturbing normal cell function. Again, it is possible that structurally related betaine compounds, such as carnitine and its derivatives, can play an important role in kidney osmoregulation. Both carnitine and betaine are: 1) mainly derived from the diet; 2) synthetized in the liver and kidney (Figs. 3 and 4); and 3) freely filtered in the kidney and mostly reabsorbed from the glomerular filtrate via active Na^+-dependent transporter localized at the proximal tubule. In the case of a well balanced diet, endogenous sources are thought to contribute modestly to carnitine or betaine pools.

Besides the liver, the kidney is the major site of carnitine and betaine biosynthesis [Gurder and Wagner, 1990; Moeckel and Lien, 1997]. Moreover, human kidney, unlike rat kidney, contains all the four enzymatic steps needed to synthesize carnitine from 6-N-trimethyllysine. As shown by Rebouche and Seim [1998], the specific activities of these enzymes exceed those found in other organs. Recently, a human cDNA encoding gamma-butyrobetaine hydroxylase, the last enzyme in the biosynthetic pathway of carnitine, has been identified and the expression levels of the specific transcripts in tissues have been determined [Vaz et al., 1998]. gamma-Butyrobetaine hydroxylase mRNA expression has been detected only in kidney, liver, and brain. As expected, expression was highest in kidney and moderate in liver; brain expression was very low. The betaine synthetized in the kidney can accumulate in the medulla, and medullary concentrations of newly synthetized betaine are closely related to the hydration state of the animal [Moeckel and Lien, 1997].

Interestingly, carnitine is highly concentrated in renal cortex [Guder and Wagner, 1990], but there are no data about the site of synthesis or concentration differences between renal structures. The local and significantly high synthesis of carnitine in the kidney could be relevant from an osmoregulatory and osmoprotectant viewpoint. Stimulation of the osmolyte carnitine flux subsequent to physiological alterations of renal cell volume, subserves cell volume regulation mechanisms and macromolecule stabilization, rather than (only) lipid metabolism modulation. It is not surprising that overlapping mechanisms seem to act synergistically in kidney osmoregulation, since cells generally use several mechanisms in parallel to control cell volume.

The hypothesis that there is osmoregulatory carnitine uptake in the kidney, i.e., a carnitine transport mechanism different from that localized in the proximal tubule that is responsible for carnitine reabsorption, again derives from the parallel drawn with the osmolyte betaine.

Na^+- and H^+-dependent betaine transporters have been detected in brush-border membrane vesicles isolated from rabbit proximal tubules [Wunz and Wright, 1993]. These apical transporters may be responsible for betaine re-

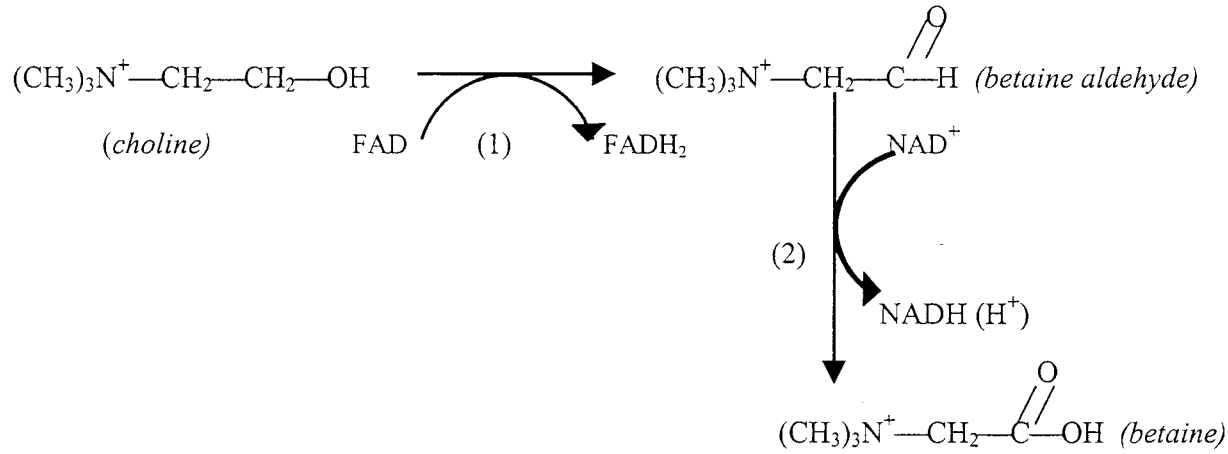


Fig. 4. Betaine synthesis. (1) Choline dehydrogenase; (2) betaine aldehyde dehydrogenase.

absorption in the nephron and the very low excretion of betaine in urine. The other transport mechanism is osmoregulatory betaine uptake in the renal medulla. A different Na^+- and Cl^+-dependent betaine transporter that plays a major role in betaine uptake in renal medullary cells has been identified on the basolateral plasma membrane. This is the [gamma]-amino-*n*-butyric acid transporter (BGT-1 mRNA) [Miyai et al., 1996]. Eight variants (divided into three main types) of the mRNA have been identified. Each type is expressed in a tissue-specific manner. Type A is detected only in kidney medulla. Type B is found in brain, liver, kidney cortex, and medulla. Type C is found in brain, kidney cortex and medulla. Hypertonicity induces all three mRNA isoforms in the Madin-Darby canine kidney [Takenaka et al., 1995; Burg et al., 1997].

Metabolic Implication of Cell Osmolarity Regulation

It is well known that cell efflux and influx of organic osmolytes are finely tuned by the osmotic pressure of the microenvironment. Several observations support the hypothesis that transmembrane efflux of organic compatible solutes is largely mediated in many cell types by a swelling-activated anion channel designated VSOAC [Strange et al., 1996; Kirk et al., 1998]. The VSOAC is ubiquitous in mammalian cells and is also present in cells of such lower vertebrates as *Xenopus laevis* and the salt water skate *Raja erinacea.*

Regulation of VSOAC activity is poorly understood. It appears that the VSOAC is sensitive to intracellular ATP levels, which suggests that the metabolic state of the cell may modify the volume sensitivity or set-point of the VSOAC. It is also sensitive to intracellular salt concentration, thus allowing cells to use electrolytes or a combination of electrolytes and organic osmolytes for the regulation of volume decrease [Strange et al., 1996]. The dependence of organic osmolyte efflux on cellular ATP level has important physiological implications. Organic osmolytes are metabolically "expensive" for cells to accumulate [Garcia-Perez and Burg, 1991]. Because these solutes represent a significant fraction of the total intracellular osmolarity and because they may play important "cytoprotective" roles, it would be advantageous to reduce their passive loss when cellular energy production is reduced. Moreover, modulation of the VSOAC by ATP levels functions as an important feedback regulatory mechanisms, in that the channel is highly permeable to such metabolic intermediates as pyruvate, the short-chain fatty acids acetate and butyrate, the ketone body beta-hydroxybutyrate, and many amino acids [Jackson et al., 1994]. These metabolites are major energy-producing components of the Krebs cycle, and their unregulated loss could disrupt cellular ATP production. Thus, the dependence of VSOAC on ATP levels is a primitive adaptation that allows cells to cope with the competing demands of volume control, maintenance of correct intracellular organic osmolyte levels, and preservation of energy metabolism [Jackson et al., 1994; Strange et al., 1996]. Maintenance of a constant volume is crucial for the optimal func-

TABLE I. Cell Volume Control of Metabolic Processes

Stimulation upon swelling and/or inhibition by shrinkage	Inhibition upon swelling and/or stimulation by shrinkage
Glycogen synthesis in hepatocytes, and muscle	Glycogenolysis
Glucokinase activity in hepatocytes	Glucose-6-phosphatase activity
Glycolysis in hepatocytes, macrophages, and lymphocytes	Glycolysis in muscle and fibroblasts
Lactate uptake in hepatocytes	Release of glutamine and alanine from muscle
Pentose phosphate shunt in hepatocytes	Proteolysis in hepatocytes
Protein synthesis in hepatocytes, mammary cells, and HeLa cells	Glutamine synthesis
Amino acid uptake	Carnitine palmitoyltransferase I activity
Glutamine breakdown in liver, lymphocytes, and macrophages	Cellular ATP concentration in hepatocytes
Glycine and alanine oxidation	Phosphocreatine concentrations in glioma cells
Urea synthesis from amino acids	Urea synthesis from NH^+_4
Glutathione (GSH) efflux	GSSG release into bile
Ornithine decarboxylase activity and expression	
RNA and DNA synthesis in HeLa cells	
Ketoisocaproate oxidation	
Acetyl-CoA carboxylase	
Lipogenesis	
Taurochocolate excretion into bile	
Respiration in glial cells and sperm	
Formation of active oxygen species in neutrophils	
Bile secretion	

tioning of cells, and swelling within certain limits for finite periods of time does not compromise cell survival. In accordance with Strange et al. [1996], it is not inconceivable that preservation of energy metabolism may take precedence over volume regulation when hypotonic stress is transient in nature or when cells are not threatened with lysis.

Cell volume modifications alter several metabolic pathways (Table I). Generally speaking, cell swelling induces the synthesis and inhibits the catabolism of proteins, glycogen, and lipids, while cell shrinkage has the opposite effect [Lang et al., 1998]. Hepatocyte swelling induced by several amino acids, notably glutamine and proline, or by hypotonicity exerts a number of anabolic and catabolic effects: stimulation of glycogen, lipid and protein synthesis, and inhibition of glycogenolysis and proteolysis [Vom Dahl and Häussinger, 1996; Guzmán et al., 1994]. Ketogenesis is also inhibited by hepatocyte swelling. Hypotonicity-induced inhibition of ketogenesis is independent of changes in the concentration of malonyl-CoA, the physiological inhibitor of carnitine palmitoyltransferase which is the key enzyme in the transport of long-chain fatty acids into the mitochondria, and therefore, in the synthesis of ketone bodies [Guzmán et al., 1994; Graf and Häussinger, 1996]. Once again, the latter effect of cell swelling on lipid metabolism caused by a decrease of extracellular osmolarity may be concomitant with the efflux of intracellular carnitine. The diminished ATP synthesis, as described above, thus controls VSOAC permeability.

CONCLUSION

There are few studies aimed specifically at evaluating the cellular functions sensitive to cell volume, namely about carnitine metabolic pathways and carnitine as an osmotically active substance. It is well established that carnitine as a carrier of acyl moieties of different chain lenghts, apart from the special long-chain fatty acids channelled to beta oxidation, intervene in cellular regulation, including membrane structure and signal transduction [Zammit, 1999]. In fact, long-chain fatty acids

and their derivatives are not only constituents of cellular structures but also they function as regulatory molecules, affecting all phases of cellular activities [Zammit, 1999; Kim, 1997]. However, the thousands of articles devoted to carnitine's metabolic role have tended not to address the issue of data that seem to indicate that carnitine functions in cell volume regulation, and in the stabilization of macromolecules. Hence this attempt to summarize the various studies leading to this concept of carnitine as an osmolyte that exerts metabolic functions.

REFERENCES

Adams SH, Esser V, Brown NF, Ing NH, Johnson L, Foster DW, McGarry JD. 1998. Expression and possible role of muscle-type carnitine palmitoyltransferase I during sperm development in the rat. Biol Reprod 59(6):1399–1405.

Arakawa T, Timasheff SN. 1985. The stabilization of proteins by osmolytes. Biophys J 47:411–414.

Bettelheim FA. 1985. Physical basis of lens transparency. In: Maisel H, editor: The ocular lens: Structure, function and pathology. New York: Marcel Dekker. p 265–295.

Beumer RR, Giffel MC, Cox LJ, Rombouts FM, Abee T. 1994. Effect of exogenous proline, betaine, and carnitine on growth of *Listeria monocytogenes* in a minimal medium. Appl Environ Microbiol 60:1359–1363.

Bremer J. 1997. The role of carnitine in cell metabolism. In: de Simone C, Famularo G, editors: Carnitine today. Heidelberg: Springer-Verlag. p 1–37.

Burg MB, Kwon ED, Kültz D. 1997. Regulation of gene expression by hypertonicity. Ann Rev Physiol 59:437–455.

Cammarata PR, Xu G-T, Huang L, Zhou C, Martin M. 1997. Inducible expression of Na+/myo-inositol cotransporter mRNA in anterior epithelium of bovine lens: Affiliation with hypertonicity and cell proliferation. Exp Eye Res 64:745–757.

Cammarata PR, Zhou C, Chen G, Singh I, Reeves RE, Kuszak JR, Robinson ML. 1999. A transgenic animal model of osmotic cataract. Part 1: Over-expression of bovine Na+/myo-inositol cotransporter in lens fiber. Invest Ophthalmol Vis Sci 40:1727–1737.

Clark ME, Zounes M. 1977. The effects of selected cell osmolytes on the activity of lactate dehydrogenase from euryhaline polychaete, *Nereis succinea*. Biol Bull 153: 468–484.

Di Mattio J, Zadunaisky J. 1981. Glucose transport into the ocular compartments of the rat. Exptl Eye Res 32: 517–532.

Garcia-Perez A, Burg MB. 1991. Role of organic osmolytes in adaptation of renal cells to high osmolarity. J Membr Biol 119:1–13.

Glaasker E, Konings WN, Poolman B. 1996. Glycine betaine fluxes in *Lactobacillus plantarum* during osmostasis and hyper- and hypo-osmotic shock. J Biol Chem 271:10060–10065.

Goldstein L, Davis-Amaral EM, Musch MW. 1996. Organic osmolytes channels: Transport characteristics and regulation. Kidney Int 49(6):1690–1694.

Graf J, Häussinger D. 1996. Ion transport in hepatocytes: mechanisms and correlations to cell volume, hormone actions and metabolism. J Hepatol 24(1):53–77.

Guder WG, Wagner S. 1990. The role of the kidney in carnitine metabolism. J Clin Chem Clin Biochem 28: 347–350.

Guzmán M, Velasco G, Castro J, Zammit VA. 1994. Inhibition of carnitine palmitoyltransferase I by hepatocyte swelling. FEBS Lett 344:239–241.

Jackson PS, Morrison R, Strange K. 1994. The volume-sensitive organic osmolyte-anion channel VSOAC is regulated by nonhydrolytic ATP binding. Am J Physiol 267 (Cell Physiol 36):C1203–C1209.

Kerner J, Hoppel C. 1998. Genetic disorders of carnitine metabolism and their nutritional management. Ann Rev Nutr 18:179–206.

Kim KH. 1997. Regulation of mammalian acetyl-coenzyme A carboxylase. Annu Rev Nutr 17:77–99.

Kirk K, Strange K. 1998. Functional properties and physiological roles of organic solute channels. Ann Rev Physiol 60:719–739.

Kispal GY, Melegh B, Alkonyi I, Sandor A. 1987. Enhanced uptake of carnitine by perfused rat liver following starvation. Biochim Biophys Acta 896:96–102.

Lang F, Busch GL, Ritter M, Völkl H, Waldegger S, Gulbins E, Häussinger D. 1998. Functional significance of cell volume regulatory mechanisms. Physiol Rev 78(1): 247–306.

McManus ML, Churchwell KB, Strange K. 1995. Mechanisms of disease: Regulation of cell volume in health and disease. New Engl J Med 333(19):1260–1266.

Mesmer OT, Lo TCY. 1990. Hexose transport properties of myoblasts isolated from patients with suspected muscle carnitine deficiency. Biochem Cell Biol 68:1372–1379.

Miyai A, Yamauchi A, Moriyama T, Kaneko T, Takenada M, Sugiura T, Kitamura H, Ando A, Tohyama M, Shimada S, Imai E, Kamada T. 1996. Expression of betaine transporter mRNA: its unique localization and rapid regulation in rat kidney. Kidney Int 50(3):819–827.

Moeckel GW, Lien YHH. 1997. Distribution of the novo synthetized betaine in rat kidney: Role of renal synthesis on medullary betaine accumulation. Am J Physiol (Renal Physiol 41) 272(41):F94–F99.

Molstad P. 1980. The efflux of L-carnitine from cells in culture (CCL 27). Biochim Biophys Acta 597:166–173.

Pessotto P, Valeri P, Arrigoni-Martelli E. 1994. The presence of L-carnitine in ocular tissues of the rabbit. J Ocul Pharmacol 10:643–651.

Pessotto P, Liberati R, Petrella O, Romanelli L, Calvani M, Peluso G. 1997. In experimental diabetes the decrease in the eye of lens carnitine levels is an early important and selective event. Exp Eye Res 64:195–201.

Poolman B, Glaasker E. 1998. Regulation of compatible solute accumulation in bacteria. Mol Microbiol 29(2): 397–407.

Rebouche CJ. 1977. Carnitine movement across muscle cell membranes. Studies in isolated rat muscle. Biochim Biophys Acta 471:145–155.

Rebouche CJ, Seim H. 1998. Carnitine metabolism and its regulation in microorganisms and mammals. Ann Rev Nutr 18:39–61.

Sandor A, Kispal GY, Melegh B, Alkonyi I. 1985. Release of carnitine from the perfused rat liver. Biochim Biophys Acta 835:83–91.

Sandor A, Kispal GY, Melegh B, Alkonyi I. 1987. Ester composition of carnitine in the perfusate of liver and in the plasma of donor rats. Eur J Biochem 170:443–445.

Sartorelli L, Ciman M, Rizzoli V, Siliprandi N. 1982. On the transport mechanisms of carnitine and its derivative in rat heart slices. Ital J Biochem 31(4):261–268.

Sartorelli L, Ciman M, Mantovani G, Siliprandi N. 1985. Carnitine transport in rat heart slices: II. The carnitine/deoxycarnitine antiport. Ital J Biochem 34:282–287.

Scholte HR, Boonman AMC, Hussaarts-Odijk LM, Ross JD, Van Oudheusden LJ, Rodrigues Pereira R, Wallenburg HCS. 1996. New aspects of the biochemical regulation of the carnitine system and mitochondrial fatty acid oxidation. In: Seim H, Löster H, editors: Carnitine. Pathobiochemical basics and clinical applications. Bochum: Ponte Press Verlags-GmbH, p 11–31.

Shaw RD, Li BUK, Hamilton JW, Shug AL, Olsen WA. 1983. Carnitine transport in rat small intestine. Am J Physiol 245:376–381.

Shennan DB, Grant A, Ramsay RR, Burns C, Zammit VA. 1998. Characteristics of L-carnitine transport by lactating rat mammary tissue. Biochim Biophys Acta 1393: 49–56.

Somero GN. 1986. Protons, osmolytes, and fitness of internal milieu for protein function. Am J Physiol 251 (20): 197–213.

Somero GN, Osmond CB, Bolis CL. 1992. Water and life. Berlin: Springer.

Stieger B, O'Neill B, Krahenbuhl S. 1995. Characterization of L-carnitine transport by rat kidney brush-border-membrane vescicles. Biochem J 309(2):643–647.

Strange K, Emma F, Jackson PS. 1996. Cellular and molecular physiology of volume-sensitive anion channels. Am J Physiol 270(39):711–730.

Takenaka M, Bagnasco S, Preston A, Uchida S, Yamauchi A, Kwon HM, Handler JS. 1995. The canine betaine-GABA (γ-amino-n-butyric acid) transporter gene. Diverse mRNA isoforms are regulated by hypertonicity and are expressed in a tissue-specific manner. Proc Natl Acad Sci USA 92:1072–1076.

Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, Tsuji A. 1998. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J Biol Chem 273(32): 20378–20382.

Vaz FM, Van Gool S, Ofman R, Ijlst L, Wanders RJA. 1998. Carnitine biosynthesis: Identification of the cDNA encoding human γ-butyrobetaine hydroxylase. Biochem Biophys Res Commun 250:506–510.

Verheul A, Rombouts FM, Beumer RR, Abee T. 1995. An ATP-dependent L-carnitine transporter in *Listeria monocytogenes* Scott A is involved in osmoprotection. J Bacteriol 177:3205–3212.

Verheul A, Wouters JA, Rombouts FM, Abee T. 1998. A possible role of ProP, ProU and CaiT in osmoprotection of *Escherichia coli* by carnitine. J Appl Microbiol 85: 1036–1046.

Vom Dahl S, Haussinger D. 1996. Nutritional state and the swelling-induced inhibition of proteolysis in perfused rat liver. J Nutr 126(2):395–402.

Wunz TM, Wright SH. 1993. Betaine transport in rabbit renal brush-border membrane vescicles. Am J Physiol (Renal Fluid Electrolyte Physiol 33) 264(33):F948–F955.

Wyn Jones RG. 1984. Phytochemical aspects of osmotic adaptation. Recent Adv Phytochemistry 18:55–78.

Yamamoto M, Turner TT. 1991. Epididymis, sperm maturation, and capacitation. In: Lipshultz LI, Howards SS, editors: Infertility in the male. St. Louis, MO: Mosby Year Book. p 103–123.

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. 1982. Living with water stress: Evolution of osmolyte systems. Science 217:1214–1222.

Zammit VA. 1999. Carnitine acyltransferase: Functional significance of subcellular distribution and membrane topology. Progress Lipid Res 38:199–224.

Zografi G, Yalkowsky SH. 1970. Potentiometric titration of monomeric and micellar acylcarnitines. J Pharm Sci 59: 798–803.