Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines[1–3]

François Blachier, Claire Boutry, Cécile Bos, and Daniel Tomé

ABSTRACT

L-Glutamate is one of the most abundant amino acids in alimentary proteins, but its concentration in blood is among the lowest. This is largely because L-glutamate is extensively oxidized in small intestine epithelial cells during its transcellular journey from the lumen to the bloodstream and after its uptake from the bloodstream. This oxidative capacity coincides with a high energy demand of the epithelium, which is in rapid renewal and responsible for the nutrient absorption process. L-Glutamate is a precursor for glutathione and *N*-acetylglutamate in enterocytes. Glutathione is involved in the enterocyte redox state and in the detoxication process. *N*-acetylglutamate is an activator of carbamoylphosphate synthetase 1, which is implicated in L-citrulline production by enterocytes. Furthermore, L-glutamate is a precursor in enterocytes for several other amino acids, including L-alanine, L-aspartate, L-ornithine, and L-proline. Thus, L-glutamate can serve both locally inside enterocytes and through the production of other amino acids in an interorgan metabolic perspective. Intestinal epithelial cell capacity to oxidize L-glutamine and L-glutamate is already high in piglets at birth and during the suckling period. In colonocytes, L-glutamate also serves as a fuel but is provided from the bloodstream. Alimentary and endogenous proteins that escape digestion enter the large intestine and are broken down by colonic bacterial flora, which then release L-glutamate into the lumen. L-Glutamate can then serve in the colon lumen as a precursor for butyrate and acetate in bacteria. L-Glutamate, in addition to fiber and digestion-resistant starch, can thus serve as a luminally derived fuel precursor for colonocytes. *Am J Clin Nutr* 2009;90(suppl):814S–21S.

INTRODUCTION

In 1973 Abidi and Mercer (1) measured concentrations of individual amino acids and peptides in the jejunal contents of the small intestine in healthy human volunteers who received a test meal containing 50 g of purified bovine serum albumin. Three hours after the test meal was ingested, they found that the jejunal luminal content of L-glutamate was quite high (2.6 mmol/L). They also found that, among amino acids contained in luminal peptides, L-glutamate was the most abundant of the 18 amino acids analyzed. In sharp contrast, after the test meal L-glutamate concentration in venous blood plasma averaged 58 μmol/L, which is among the lowest concentrations compared with those of the other amino acids (1). The fact that L-glutamate, in both free and peptide-bound forms, was very abundant in small intestinal luminal fluid was not surprising in itself when taking into account that this amino acid is the most abundant amino acid (after lysine) in the protein used in the study (bovine serum albumin). This latter protein is not unusual in its abundance of L-glutamate; indeed, it can generally be considered that L-glutamate is one of the most abundant amino acids in dietary proteins (2). In their article, Adibi and Mercer pointed out that "in view of the complexity of transport and metabolic steps interposed in the process of amino acid movement from the gut into the periphery, the absence of a precise relationship (between amino acids in the alimentary protein and plasma concentration) is understandable" (p 1593). Nonetheless, despite this complexity of individual events that allow amino acids in alimentary and endogenous proteins to be used by the body (particularly at the intestinal level), a relatively clear picture has emerged from human and animal studies performed in recent decades.

This review focuses on the metabolism of L-glutamate in the epithelial cells of both the small and large intestine and on the physiologic functions related to L-glutamate metabolism. The metabolism and function of L-glutamate in intestinal epithelial cells cannot be described without considering L-glutamine, because these 2 amino acids possess a partially common metabolic fate. We have differentiated effects of L-glutamine on intestinal epithelial cells that can be mimicked by L-glutamate from effects that can be obtained exclusively with L-glutamine. From experiments performed with enterocytes isolated at different developmental stages, it appears that some L-glutamate and L-glutamine metabolic pathways are exclusively operative in a short period of time after birth. Last, although the metabolism and associated functions of L-glutamate and L-glutamine have been much less studied in colonic epithelial cells (colonocytes) than in small intestinal epithelial cells (enterocytes), there are indications of a complex metabolic interplay in colonocytes between substrates of luminal bacterial origin and substrates such as L-glutamate and L-glutamine of blood plasma origin.

[1] INRA, CRNH-IdF, UMR914 Nutrition Physiology and Ingestive Behavior, Paris, France (FB, C Boutry, and C Bos), and AgroParisTech, CRNH-IdF, UMR914, Paris, France (DT).

[2] Presented at the "100th Anniversary Symposium of Umami Discovery: The Roles of Glutamate in Taste, Gastrointestinal Function, Metabolism, and Physiology," held in Tokyo, Japan, September 10–13, 2008.

[3] Address correspondence to F Blachier, UMR 914 INRA–AgroParisTech Nutrition Physiology and Ingestive Behavior, AgroParisTech, 16 rue Claude Bernard, F-75005 Paris, France. E-mail: francois.blachier@agroparistech.fr.

First published online July 1, 2009; doi: 10.3945/ajcn.2009.27462S.

Am J Clin Nutr 2009;90(suppl):814S–21S. Printed in USA.

L-GLUTAMATE AS AN OXIDATIVE SUBSTRATE IN SMALL INTESTINE EPITHELIAL CELLS

L-Glutamate and glutamate-containing peptides in the lumen of the small intestine originate not only from alimentary and endogenous proteins through the preliminary action of protease/peptidase activities but also from alimentary free L-glutamate, which is present in significant amounts in alimentary products (2) and from monosodium glutamate (MSG) used as a food additive for its flavor-enhancing property. The Na^+-dependent high-affinity X^-_{AG} system and/or the low-affinity B^0 system have been shown to be involved in the transport of L-glutamate from the lumen into the intestinal epithelial cells (3). After partial degradation of luminal peptides through the activities of brush-border-associated peptidase activities, di- and tripeptides (including those containing L-glutamate) enter enterocytes through PepT1 proton-coupled peptide transporters (4) and release L-glutamate by the action of cytosolic peptidase activities (5). Then, free L-glutamate is extensively metabolized by enterocytes in various pathways, including those involved in enterocyte energy production. A number of studies have shown that a large proportion of L-glutamate is metabolized during its transcellular journey through enterocytes. In healthy volunteers, it has been shown that nearly all of the enterally delivered L-glutamate is removed by the splanchnic bed on the first pass (6, 7). In pigs, virtually all of the enteral L-glutamate is metabolized by the gut during absorption (8). In a 7-kg piglet, an increase in L-glutamate concentrations was observed in portal and arterial blood plasma when the basal milk formula, administered enterally at a rate of 510 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, was supplemented with MSG (1250 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) (9). Similarly, in larger (60 kg) pigs, transient portal and arterial hyperglutamatemia was observed when the diet (ie, 800 g meal containing 142 g casein) was supplemented with 10 g MSG (10), indicating that, in these experimental situations, very large doses of L-glutamate may exceed the intestinal capacity to catabolize this amino acid. It was determined that dietary L-glutamate is the most important contributor to mucosal oxidative metabolism in piglets (11). The pig represents a useful experimental model, because its intestinal metabolism and physiology are not vastly different from that of humans (12). It seems likely that part of L-glutamate that enters into enterocytes from either the luminal or the basolateral direction is sequestered in an intracellular pool, because L-glutamate concentration in enterocytes and intestinal mucosa has repeatedly been reported to be high (13, 14).

There is no doubt that oxidation represents the main metabolic fate of L-glutamate within enterocytes. The pioneering work of Windmueller and Spaeth (15) showed that carbon dioxide is the major metabolic end product of L-glutamate metabolism in the rat, regardless of whether it is supplied from the lumen or the arterial blood supply. Ashy and Ardawi (16) reported that, when used at the same concentration, L-glutamate and L-glutamine were each able to increase basal oxygen consumption to a similar extent in isolated human enterocytes. The metabolic steps involved in L-glutamate oxidation in enterocytes first involve transamination with oxaloacetate to produce α-ketoglutarate and L-aspartate (**Figure 1**). L-Glutamate can also be transaminated in the presence of pyruvate to produce L-alanine and α-ketoglutarate. Transamination appears to be the principal route by which L-glutamate is converted to α-ketoglutarate in enterocytes, because these cells have little capacity for the conversion of L-glutamate into α-ketoglutarate (and ammonia) through glutamate dehydrogenase (17). α-Ketoglutarate produced by transamination can then enter the mitochondria, and its metabolism via the tricarboxylic acid (TCA) cycle produces reduced coenzymes (NADH, $FADH_2$) used by the mitochondria for ATP synthesis. L-Aspartate produced by L-glutamate transamination can enter mitochondria and can also be oxidized in the TCA cycle (18), thus representing another oxidative fuel for enterocytes. L-Glutamate and L-glutamine are similarly oxidized by enterocytes (10). However, for L-glutamine, the amino acid must

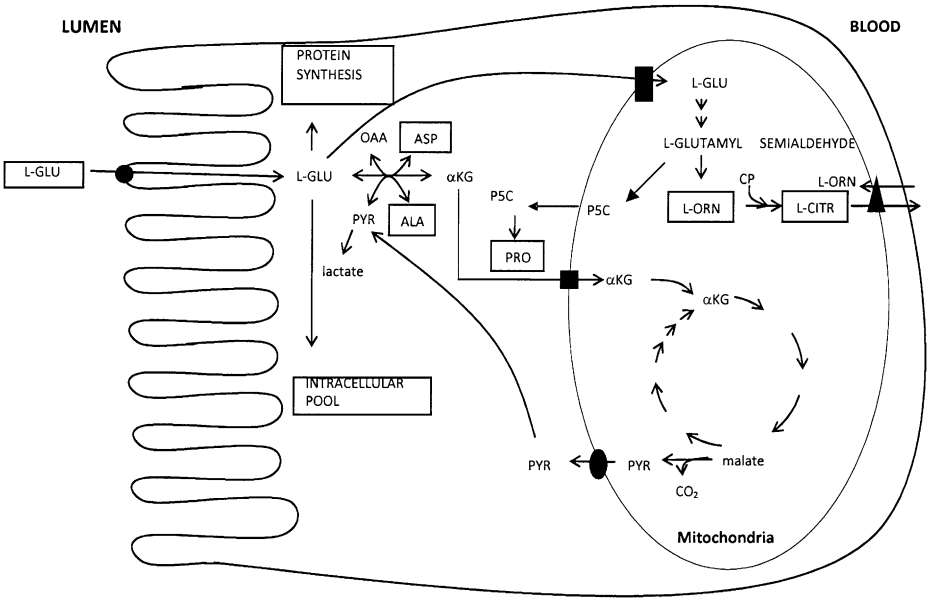

FIGURE 1. Schematic view of the metabolism of L-glutamate (L-GLU) in small intestine absorptive epithelial cells. This schema is mainly intended to represent amino acids produced within enterocytes from L-glutamate as well as the conversion of L-glutamate to α-ketoglutarate (αKG). OAA, oxaloacetate; ASP, L-aspartate; PYR, pyruvate; ALA, L-alanine; L-ORN, L-ornithine; L-CITR, L-citrulline; CP, carbamoylphosphate; P5C, pyrroline-5-carboxylate; PRO, L-proline.

first enter the mitochondria and be degraded to ammonia and L-glutamate by the phosphate-dependent glutaminase, which is abundant in enterocyte mitochondria in both villus and crypt cells (19). L-Glutamate that arises within the mitochondria may be exported into the cytosol, where it is metabolized into α-ketoglutarate before reentering the mitochondria and the TCA cycle (20). In this way, L-glutamate and L-glutamine may be equally effective as fuels for energy production within enterocytes.

When both L-glutamate and L-glutamine are simultaneously presented to enterocytes, L-glutamate is able to inhibit L-glutamine utilization and oxidation (10). This sparing effect of L-glutamate over L-glutamine is presumably dependent on the relative concentrations of both amino acids inside enterocytes.

ATP production and utilization are particularly active in enterocytes. Although the gastrointestinal tract represents only ≈5% of body weight, it is responsible for ≈20% of whole-body oxygen consumption (21, 22). The intestinal epithelial cells have high energy demands (23), due to the rapid renewal of the epithelium every few days (24, 25) and sodium extrusion at the basolateral membranes through the activity of Na/K ATPase (26).

L-Glutamine, but not L-glutamate, can be used for purine and pyrimidine synthesis (27) and can serve as a precursor in *N*-acetylglucosamine and *N*-acetylgalactosamine synthesis, which is involved in intestinal mucin synthesis (28). Due to a very low glutamine synthetase activity in small intestine mucosa (29), the capacity for de novo synthesis of L-glutamine from L-glutamate is very limited. This modest glutamine synthetase expression is located primarily in the crypt region of the small intestine, where cell mitosis is active (30).

L-GLUTAMATE AS A PRECURSOR FOR OTHER AMINO ACIDS AND PROTEIN SYNTHESIS IN THE INTESTINAL MUCOSA

L-Glutamate, in addition to being used with other amino acids for protein synthesis within the intestinal mucosa (31), can also be used by enterocytes to produce other amino acids, including L-aspartate (10), L-alanine (32), L-proline (34), L-ornithine (33), and L-citrulline (34). The latter 2 amino acids are not present in proteins but play important roles in interorgan metabolism. As indicated in Figure 1, L-aspartate and L-alanine are produced by transamination of L-glutamate in the presence of oxaloacetate and pyruvate, respectively (35). L-Glutamate can also serve as a precursor for the stepwise production of L-ornithine in mitochondria. Then, L-ornithine can serve as a substrate for L-citrulline production. Interestingly, it has been proposed that postabsorptive plasma L-citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans (36). L-Citrulline that is released into the portal vein is believed to pass through the liver without significant uptake and used by the kidneys in the de novo synthesis of L-arginine in humans (37) and other mammals (38). In studies in rats, the pharmacologic inhibition of intestinal L-citrulline synthesis produces severe growth retardation (39). L-Citrulline can be synthesized in enterocytes from both L-glutamine and L-glutamate (15). However, because glutaminase is highly expressed in small intestinal epithelial cells, whereas glutamate dehydrogenase is not, L-glutamine utilization in enterocytes (40), but not that of L-glutamate (41), produces substantial quantities of ammonia.

Both L-glutamate and L-glutamine are effective precursors for the production of other amino acids (10, 15). There is evidence that L-glutamine and L-arginine work synergistically in L-citrulline production by enterocytes (42). Indeed, in enterocytes, L-arginine is a better precursor for L-ornithine production than is L-glutamine (13), and L-glutamine, through the catalytic activity of mitochondrial glutaminase, produces ammonia, which serves as a precursor for carbamylphosphate production (34). This latter metabolite is a cosubstrate for L-citrulline synthesis.

L-GLUTAMATE AS A PRECURSOR OF GLUTATHIONE AND ACETYLGLUTAMATE IN INTESTINAL MUCOSA

Together with L-cysteine and glycine, L-glutamate is the precursor for the synthesis of glutathione in the enterocyte cytosol (**Figure 2**). This pathway is probably more limited by L-cysteine than by L-glutamate availability. Studies in pigs suggest extensive utilization of dietary cysteine by the intestine (43). It is also metabolized by isolated enterocytes (44). It has been reported that in fed piglets, mucosal glutathione is derived largely from the direct metabolism of enteral L-glutamate (45). The ratio of reduced to oxidized glutathione in enterocytes is an important measure for both the determination of the intracellular redox status (46) and for the cell's capacity to control intracellular concentrations of both oxygen-reactive and nitrogen-reactive species (47). Indeed, it has been shown that the pharmacologic inhibition of mucosal glutathione synthesis is associated with alterations of intestinal functions that can be prevented by giving glutathione monoester orally (48). In addition to mucosal glutathione synthesis, human enterocytes are capable of extracellular glutathione uptake (49).

L-glutamate in enterocytes is also involved in a quantitatively minor metabolic pathway, ie, *N*-acetylglutamate synthesis. *N*-acetylglutamate is produced from acetyl-coenzyme A and L-glutamate by *N*-acetylglutamate synthase. This latter enzyme was detected in both intestinal mucosa (50) and in enterocytes (51). It can be activated by L-arginine and is found within enterocyte mitochondria, together with carbamoyl synthetase 1 and ornithine carbamoyltransferase (42). Because *N*-acetylglutamate is an allosteric activator of carbamoyl synthetase 1, this L-glutamate–derived metabolite is believed to play a role in the capacity of enterocytes to produce L-citrulline.

ENTEROCYTE L-GLUTAMATE, L-GLUTAMINE, AND L-ARGININE METABOLISM IN RELATION TO DEVELOPMENTAL STAGE

As described above, the metabolism of L-glutamate, L-glutamine, and L-arginine is deeply interwoven. Some metabolic pathways used by these amino acids have been shown to be constitutively maintained in enterocytes during development, whereas others are deeply modified by developmental stage. In humans, for example, it has been shown that, in enterally fed, preterm infants, L-glutamate is an important energy source for the splanchnic area (52). Enterocytes that are isolated from piglet small intestine at birth display a high capacity for L-glutamine oxidation (53), indicating that this amino acid can be used as a fuel at this stage of development. Furthermore, enterocytes isolated from newborn pigs are able to convert L-glutamine into L-citrulline and L-arginine (54). In contrast, L-arginine is very

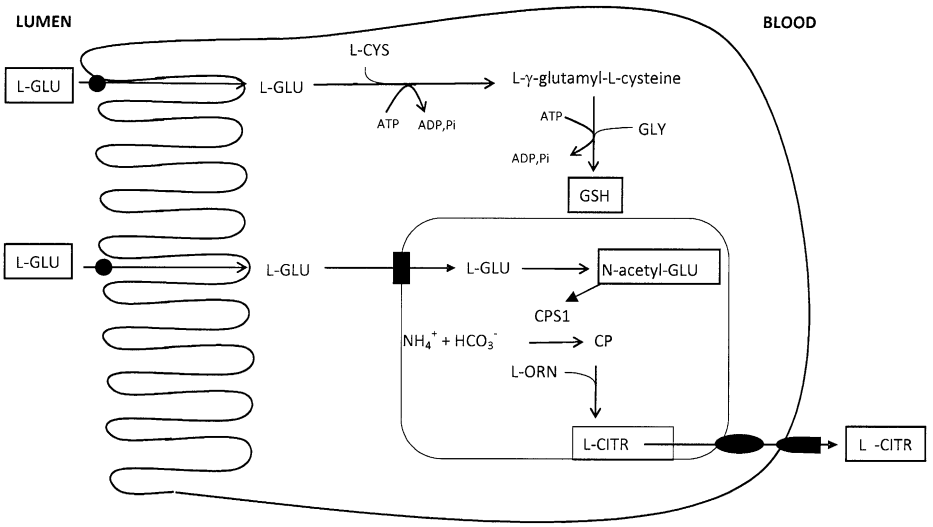

FIGURE 2. Schematic view of the conversion of L-glutamate (L-GLU) into glutathione and *N*-acetylglutamate (N-acetyl-GLU) in small intestine absorptive epithelial cells. This schema also represents the activation of carbamoylphosphate synthetase 1 (CPS1) by *N*-acetylglutamate for L-citrulline (L-CITR) production. L-CYS, L-cysteine; GLY, glycine; L-ORN, L-ornithine; CP, carbamoylphosphate.

little used in the arginase and nitric oxide synthase pathways, indicating that, at this developmental stage, metabolism is orientated toward L-arginine production (55). At birth, there is a relatively high ornithine decarboxylase activity (ODC), which falls after 2 d. However, the flux of L-ornithine through ODC is relatively modest (56). The polyamines, which can also be imported from the extracellular medium into enterocytes, are known to be implicated in the process of cell division and differentiation, which is very intense after birth in the intestinal mucosa of humans (57) and other mammals (58). The capacity of epithelial cells to synthetize L-arginine from L-glutamine and L-glutamate may reflect the high content in milk proteins of glutamate and glutamine and the relatively low content of arginine (59). In this context, in premature or low-birth-weight infants, a moderate and transient hyperammonemia that is reversible with L-arginine is often observed (60). This hyperammonemia is due to a low plasma concentration of L-arginine and L-ornithine. It has been proposed that the slight hyperammonemia in low-birth-weight infants may be due to an incomplete repletion of L-arginine in the liver urea cycle, but it is not known if the capacity of intestine for L-arginine synthesis is altered in such infants.

In suckling piglets, it was observed that, soon after birth, the capacity of enterocytes to convert L-glutamine to L-citrulline is severely decreased, compared with the situation at birth (54). In contrast, L-glutamine still represented a major oxidative substrate for enterocytes in these animals. Indeed, in suckling piglet enterocytes, L-glutamine was ≈8 times more rapidly oxidized than in enterocytes isolated from weaned pigs (53). In addition, L-arginine utilization in L-ornithine– and nitric oxide–producing pathways was markedly increased, indicating that a pseudo-urea cycle is operative in suckling piglet enterocytes (54, 55). Last, ornithine decarboxylase activity in enterocytes was severely diminished, which suggests a dependence of enterocytes on luminal polyamines in these animals. Milk is relatively rich in polyamines in both humans (61) and other mammals (62). The metabolic situation observed in suckling piglet enterocytes is transient, because other modifications are observed between the suckling and postweaning periods. Indeed, in enterocytes isolated at that latter stage of development, the capacity of enterocytes to convert L-citrulline into L-arginine was lost, and the capacity of the cells to convert L-arginine into nitric oxide and L-ornithine was greatly increased (54, 55). Because nitric oxide production in the small intestinal mucosa is required for the maintenance of epithelial integrity and the modulation of epithelial permeability (63, 64), the increased expression of nitric oxide synthase during development may be related to the acquisition of new intestinal functions.

L-GLUTAMATE AND L-GLUTAMINE METABOLISM IN COLON EPITHELIAL CELLS

It is well known that there are important differences between the luminal environment in the small and large intestines. The colonic epithelium (like the small intestinal epithelium) is a structure in rapid renewal (65). This process of constant renewal, together with the activity of colonocytes in transporting water and electrolytes, makes the epithelial colonic cells high energy consumers (66). It is therefore important to identify the oxidative substrates of both blood and luminal origin. The luminal contents that face the epithelium are characterized by high quantities of bacteria together with a high concentration of bacterial metabolites (67), some of which are known fuels, and others which are suspected of being "energy metabolism troublemakers" when present in excess (68). Another important characteristic of the colonic epithelium is that, except for a very short period after birth, there is little or no transfer of amino acids from the lumen to portal blood (69). Under such circumstances, amino acids (including L-glutamate and L-glutamine) must be taken into colonocytes from arterial blood. Colonic differentiated epithelial cells can use L-glutamine from the blood plasma as an oxidative substrate (70). L-Glutamine is first converted into L-glutamate and ammonia by the mitochondrial enzyme glutaminase, and then into α-ketoglutarate, mainly by transamination, followed by entry into the TCA cycle (20) (**Figure 3**).

Colonocytes can also use luminal organic acids generated from microbial activity, including short-chain fatty acids, as oxidative substrates (67). Dietary substrates for short-chain fatty acid production are mainly dietary fiber, resistant starch, and proteins (71). Although alimentary protein digestion followed by amino acid and oligopeptide absorption by the small intestine is efficient (72), substantial amounts of nitrogenous compounds of both exogenous and endogenous origin enter the large intestine through the ileocecal junction. In humans, this nitrogenous material, consisting mainly of proteins and peptides (73), is quantitatively related to the amounts of ingested proteins (74) and represents between 6 and 18 g/d (75). The first event in colonic protein degradation is hydrolysis of proteins and polypeptides by proteases and peptidases, which results in peptide and amino acid release, followed by the production of numerous bacterial metabolites.

There is no doubt that several of these compounds can exert effects (beneficial and deleterious) on colonic epithelial cell metabolism and function. The study of these effects has drawn little attention. These effects are likely to depend on factors such as luminal concentrations (which can be modified by diet), colonic transit time, detoxifying capacity of epithelial cells in response to increased quantities of deleterious compounds, and cellular metabolic utilization of the luminal metabolites and their effects on colonocyte intermediary and oxidative metabolism (68).

In the large intestinal lumen, L-glutamate released from proteins and peptides is the precursor for acetate and butyrate production (67), but the relative contributions of L-glutamate and alimentary polysaccharides to acetate and butyrate production have not been determined. Glutamine synthetase activity, in contrast to what is found in small intestinal mucosa, is relatively high in the large intestinal mucosa (29). Recently, we found that ATP-dependent glutamine synthetase activity in rat isolated colonocytes is 10 times more than the activity measured in isolated enterocytes (F Allek and F Blachier, unpublished data, 2009). Because the L-glutamine–degrading enzyme glutaminase is also highly expressed in colonocytes (76), this raises the open question of the physiologic meaning of the expression within the same cells of L-glutamine–synthesizing and –degrading enzymes. Because ammonia at concentrations that can be found in the colonic lumen inhibits short-chain fatty acid oxidation in colonic epithelial cells (77, 78), it can be speculated that cytosolic glutamine synthetase activity, which converts L-glutamate and ammonia into L-glutamine, may represent a way to reduce the intracellular concentration of ammonia during its transfer from the lumen to the bloodstream. Carbamoylphosphate synthetase 1 and ornithine transcarbamylase activities can be measured in rat colonocyte mitochondria (79). Because ammonia can increase the conversion of L-arginine into L-citrulline in colonocytes (80), it is likely that this metabolic pathway may contribute to control intramitochondrial ammonia concentrations, and thus its effect on colonocyte short-chain fatty acid oxidation. Glutamine synthetase activity in colonocytes may also correspond to a fine tuning of intracellular L-glutamine concentrations in colonocytes, because this latter amino acid has been found in in vitro experiments to be implicated in protein synthesis (81) and in the control of cytokine-induced apoptosis (82).

From available data, it thus appears that energy production in colonocytes is dependent on a complex metabolic interplay between substrates of both colonic bacterial origin and from blood plasma, and that L-glutamate is likely to intervene in such a process. Utilization of L-glutamate in colonocytes is not restricted to energy metabolism. Both de novo glutathione synthesis and transport occur in colonocytes (83). Furthermore, L-glutamine metabolism in isolated colonocytes through the conversion into L-glutamate gives rise to a net production of L-aspartate, L-alanine, and lactate (84), which suggests a role of colonic epithelial cells for interorgan metabolism.

The capacity of colonocytes to use L-glutamine is high in colonocytes but remains smaller than in enterocytes (85). Accordingly, the phosphate-dependent glutaminase activity is weaker in colonocytes than in enterocytes (86). It is worth noting that

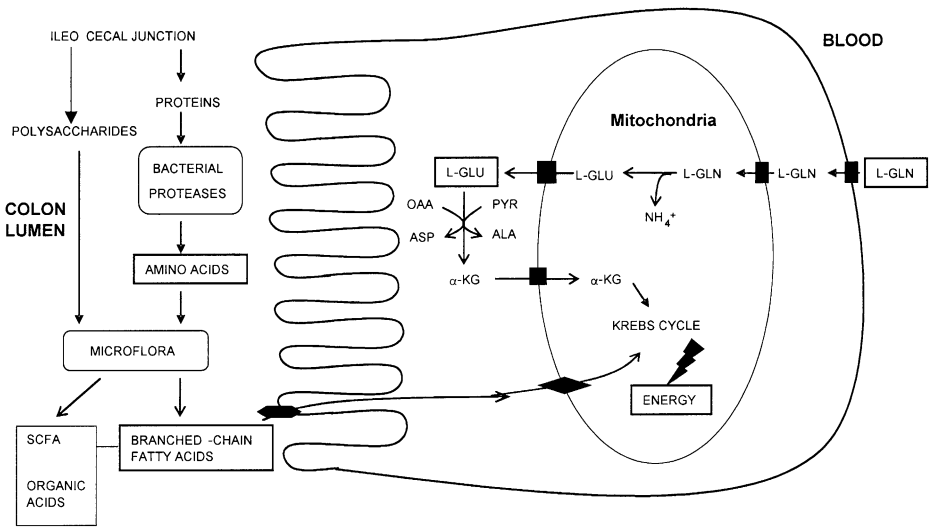

FIGURE 3. Schematic view of the production of energy in large intestine absorptive epithelial cells from plasma L-glutamate (L-GLU) and from luminal bacterial metabolites. These metabolites are produced from polysaccharides and from amino acids. Among amino acids, glutamate can serve as a precursor for butyrate and acetate production, which are oxidative substrates for colonocytes. SCFA, short-chain fatty acids; L-GLN, L-glutamine; OAA, oxaloacetate; ASP, L-aspartate; PYR, pyruvate; ALA, L-alanine; α-KG, α-ketoglutarate.

the metabolic products that derive from L-glutamine metabolism in colonocytes are also produced in enterocytes. However, unlike what is observed in enterocytes (13), colonocytes isolated from rats and pigs produce more L-aspartate than L-alanine from L-glutamine (66, 84). This coincides with a higher activity of aspartate aminotransferase than alanine aminotransferase in colonocytes (87). (Other articles in this supplement to the Journal include references 88–116.)

All authors participated equally in the writing of the manuscript. The authors' travel expenses associated with participation in the symposium and an honorarium were paid by the conference sponsor, the International Glutamate Technical Committee, a nongovernmental organization funded by industrial producers and users of glutamate in food. The authors declared no conflicts of interest in the material presented in this paper.

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