Role of the Gut in the Amino Acid Economy of the Host

Peter J. Reeds, Douglas G. Burrin, Barbara Stoll and Johannes B. van Goudoever

USDA/ARS Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Tex., USA

The intestinal tissues, especially the small intestinal mucosa, have a high rate of protein synthesis and energy expenditure. Because a substantial proportion of mucosal protein synthesis is probably devoted to secretion into the lumen, the intestine is in a permanent state of positive protein balance. In addition to the impact that it has on whole body protein turnover, the gut may also play a crucial role in amino acid metabolism. It seems likely that glutamate, glutamine, and aspartate are the dominating sources of carbon for mucosal energy generation, and it appears that dietary amino acids, as opposed to systemic amino acids, may be the more important in this respect. Moreover, there is increasing evidence that the small intestinal mucosa is a nutritionally significant site of dietary essential amino acid catabolism, at least in young mammals. Finally, the small intestinal mucosa is a key site of amino acid biosynthesis and other metabolic transformations. In this chapter, we discuss aspects of all these functions, starting with the quantification of systemic amino acid bioavailability, and passing to the relative importance of intestinal protein turnover, secretion and amino acid metabolism to the amino acid requirements of the host.

Protein Digestibility and Amino Acid Bioavailability

The traditional approach to the assessment of protein digestibility has been to relate the intake of protein and the output of fecal nitrogen. However, while this approach is eminently practical, it gives a substantially misleading impression of
Role of the Gut in the Amino Acid Economy of the Host

Table 1. First-pass splanchnic extraction of amino acids in the human

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Percent of intake metabolized</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>26 ± 7</td>
<td>4–8</td>
</tr>
<tr>
<td>Lysine</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>39</td>
<td>9, 10</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Glutamine</td>
<td>53</td>
<td>12</td>
</tr>
<tr>
<td>Glutamate</td>
<td>88</td>
<td>13</td>
</tr>
</tbody>
</table>

the digestive process. This is because of the major amino acid transformations that occur in the large bowel. A more informative approach to this problem is to compare the flow of amino acids at the terminal ileum with the amino acid intake. On the presumption that amino acid absorption from the colon is negligible, this method defines the maximum likely availability of the amino acids for extramucosal metabolism. Even so, the amino acids exiting the ileum are a mixture of undigested dietary protein and proteins that have been secreted by the organism. Thus the apparent ileal digestibility systematically underestimates the true digestion of the dietary proteins to the degree that endogenous proteins contribute to the terminal ileal digesta.

Over the last decade, isotopic approaches involving the labeling of either the animal [1] or the diet [2] with 15N have been used to determine the true digestibility of dietary protein. Although each approach has its own drawbacks, in general these studies (discussed by Fuller and Reeds [3]) suggest that the true digestibility of many proteins often exceeds 90%. It follows that under many circumstances the majority of the protein lost from the ileum is derived from the host. That being so, ileal amino acid outflow should be viewed as part of the amino acid requirement rather than a reflection of protein digestibility.

However, it is now clear that the disappearance of dietary amino acids from the lumen of the small intestine overestimates their availability to the peripheral tissues of the host. First of all, published data on humans (Table 1) show that the first pass splanchnic extraction of some essential amino acids can be as high as 50% of the enteral input [9], and for glutamate the splanchnic extraction is >80% [13]. These observations contrast with the fate of digested dietary carbohydrate, which can have a systemic availability of >90% of intake [14]. Moreover, although the splanchnic extraction of dietary amino acids reflects utilization by the intestine and liver, intestinal essential amino acid metabolism accounts for >70% of splanchnic metabolism [15–17]. Comparisons of the appearance of amino acids in the portal circulation with amino acid intake [18–21] largely confirm this conclusion.

Results from studies in pigs [19, 22–25], the species for which most data are available, are summarized in Figure 1. These reveal several interesting points
Role of the Gut in the Amino Acid Economy of the Host

Fig. 1. Portal availability of amino acids in pigs. Data are summarized from references 19, 22, 23, and 24.

that have a bearing on the identification of the factors, discussed below, that regulate the degree to which the intestine modifies amino acid availability. First, the portal outflow varies widely among amino acids, and the portal balance of dietary threonine is consistently lower than that of other essential amino acids. This implies that, regardless of the amino acid composition of the dietary protein, threonine may often be the first limiting essential amino acid for the growth of peripheral tissues. Second, nutritionally significant quantities of dietary glutamate and aspartate rarely appear in the portal blood. There is also a net extraction of glutamine across the gut. Apart from indicating the extensive utilization of these three amino acids in the intestine [24, 26, 27], the result also highlights the fact that systemic glutamate, aspartate, and glutamine are derived almost exclusively from synthesis within the body. Third, some amino acids either appear in quantities that are similar to the amounts ingested in dietary protein (arginine and tyrosine), or greatly exceed them (alanine). This is compatible with the proposition that the intestinal tissues are a nutritionally significant site of arginine, tyrosine and, especially, alanine synthesis.

These lines of evidence suggest, therefore, that intestinal metabolism has a material effect on the availability of dietary amino acids for the support of growth, reproduction, lactation, and the maintenance of health, and that the pattern of amino acids that emerges in the portal vein can be markedly different from that found in the ingested dietary protein. At the very least, this general observation has a critical effect on the development of models of amino acid requirements.
Role of the Gut in the Amino Acid Economy of the Host

Table 2. Relative rates of visceral utilization of dietary and arterial amino acids in milk-fed piglets

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>First-pass utilization of dietary amino acid</th>
<th>Utilization of mesentric arterial amino acid flux</th>
<th>Total utilization of whole body flux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/kg/h percent intake</td>
<td>µmol/kg/h percent input</td>
<td>µmol/kg/h percent of intake</td>
</tr>
<tr>
<td>Threonine</td>
<td>205 61</td>
<td>10 0.1</td>
<td>215</td>
</tr>
<tr>
<td>Lysine</td>
<td>135 32</td>
<td>229 8</td>
<td>374 46</td>
</tr>
<tr>
<td>Leucine</td>
<td>149 33</td>
<td>226 16</td>
<td>375 52</td>
</tr>
<tr>
<td>Phe. Alanine</td>
<td>54 40</td>
<td>21 7</td>
<td>75 41</td>
</tr>
<tr>
<td>Glutamate</td>
<td>562 96</td>
<td>136 11</td>
<td>700 36</td>
</tr>
<tr>
<td>Glutamine</td>
<td>147 67</td>
<td>191 21</td>
<td>338 26</td>
</tr>
</tbody>
</table>

1 The value for whole body flux includes intestinal utilization.
2 Data are from Stoll et al. [22].
3 Data are from van Goudoever et al. (unpublished).
4 Data are from Stoll et al. [29].
5 Data are from Reeds et al. [24].
6 Data are from Stoll et al. [27].

Pathways of Amino Acid Utilization by the Intestine

Before passing to a discussion of the metabolic processes that are important for the phenomena illustrated in Figure 1, it is important to consider a central feature of gut amino acid metabolism – that is, that the intestinal mucosa receives considerable quantities of nutrients directly from two sources, the lumen (and hence dietary and secreted protein) and the mesenteric artery. Thus the observations in Figure 1 could reflect a combination of different factors, each of which has a somewhat different metabolic implication. First, the amino acids could be metabolized immediately after being transported into the villous epithelial cells. Alternatively, it is possible that absorption from the small intestine into the portal circulation is 100% efficient (as indicated by some results in sheep [21]), but that there is simultaneous removal of arterial amino acids. In the event, studies of intestinal amino acid utilization with intravenous and intragastric infusions of amino acid tracers [17, 20, 22, 24, 25] show that, even in the fed state, there is both first-pass mucosal metabolism of dietary amino acids and simultaneous uptake of amino acids from the mesenteric arterial circulation by the portal drained viscera. Data from our experiments in young pigs receiving milk-based diets [22, 24, 27-29] (Table 2), as well as data obtained in sheep [20], suggest that the relative contributions of the arterial and dietary sources vary among amino acids. The critical and functionally important questions are: What
Role of the Gut in the Amino Acid Economy of the Host

pathways consume these amino acids, and what is their significance to host metabolism as a whole?

Protein Synthesis, Secretion, and Amino Acid Availability

One of the earliest observations that emerged from the initial application of stable isotopic tracers to the study of metabolism [30] was that the rate of protein synthesis in the intestine is particularly high. This observation has been confirmed repeatedly in all the commonly investigated mammalian species [31–34]. Indeed, the data in Table 2 are a clear indication of the substantial contribution of intestinal amino acid utilization to that of the whole body. However, while this general observation is true, certain features of mucosal metabolism complicate the interpretation of measurements of mucosal protein synthesis and turnover.

The first problem relates to quantification. In the fed state, because of the influx of unlabeled amino acids from the diet, tracer amino acids taken up from the circulation are substantially diluted in the mucosal free amino acid pool. Furthermore, under these circumstances, the degree of intracellular isotopic dilution varies along the intestine (Fig. 2), as well as along the villus [35]. The determination of the true isotopic enrichment of the protein synthetic precursor in the intestine is therefore particularly difficult [36, 37]. Second, as we illustrate in Table 2, the intestinal tissues utilize both luminal and arterial amino acids, and incorporate both sources into their constitutive proteins [29, 34, 35]. Unfortunately, the relative contributions of luminal and arterial amino acids remain uncertain, and, as indicated by the results in Figure 2, the relative contributions of luminal and systemic amino acids to mucosal protein synthesis may well vary with the region of the intestine. Moreover, recent evidence from experiments in which different isotopologs of the same amino acid were given intravenously and enterally [29, 34], as well as evidence from the equilibrium labeling of the precursor forms of brush border enzymes [33, 37], suggests that there is kinetic channeling of arterial amino acids to mucosal protein synthesis. In other words, there is a greater likelihood of a newly transported arterial amino acid being incorporated into protein than there is for a luminal amino acid. The structural and mechanistic basis of this compartmentation remains unknown, but, as we emphasize below, such phenomena appear to be a characteristic of mucosal substrate metabolism.

The second problem of interpretation arises from the nature of the end products of protein synthesis in the mucosa. With the exception of the liver and pancreas, protein synthesis in most other organs and tissues is largely devoted to the renewal and accretion of cellular protein. However, in the intestine this is not so, and a significant portion of the protein synthesized is lost to the lumen, either by true secretion or by what is in effect attrition at the brush border membrane, the proteins of which appear to have a higher fractional rate of protein synthesis than mucosal protein as a whole [36]. The high contribution of secretion to overall protein synthetic activity in the mucosa has at least
two implications. First, the difference between intestinal protein accretion and synthesis cannot be simply interpreted in terms of intracellular proteolysis, so that whether intracellular protein degradation in the mucosa is or is not high, remains uncertain [38]. It is more likely that the difference between protein synthesis and accretion in the gut represents the secretory component, so that kinetically the small intestine is essentially in a permanent state of net protein synthesis.

In Table 2, we show that the total utilization of lysine, leucine, and phenylalanine by the portal drained viscera accounted for >40% of the whole body amino acid flux. Similar results have been obtained in sheep [20]. However, this estimate was calculated from measurements of the unidirectional utilization of tracer amino acids. It therefore overestimates the net utilization of amino acids in secretory protein synthesis in the same way that measurements of total protein synthesis at other sites overestimate protein deposition. It is, of course, the net protein loss that directly affects amino acid requirements, and this ultimately depends on the balance between the rate of secretion and the degree to which the secreted proteins are subsequently digested and reabsorbed.

In general, there must be a high degree of reabsorption, because the total protein losses indicated by the data in Table 2 approach the magnitude of the amino acid intake of the animals, and such losses could not be sustained for anything other than a short period of time. Unfortunately, the degree of recycling has not been formally quantified, at least in any detail [3]. The simplest approach – that is, the measurement of ileal protein loss under protein-free feeding conditions – indicates that endogenous protein losses lie between 0.05 and 0.15 g protein/kg-day [1, 39], but it seems likely that these values
Role of the Gut in the Amino Acid Economy of the Host

substantially underestimate the losses under practical conditions. However, it is possible to calculate from information obtained from studies with prolonged $^{15}$N-amino acid infusions in animals [1, 40, 41], or from the ingestion of $^{15}$N-labeled diets in human beings [42], that, per kilogram of dry matter intake, between 25 and 30 g of secreted protein exits the terminal ileum. In an adult human consuming a protein-containing, weight maintenance diet, this translates into a net protein loss to the colon of about 0.2 g/kg-day, considerably in excess of that determined under conditions of protein-free feeding (0.05 g/kg-day) [39]. This is approximately 5% of whole body protein turnover. More importantly, the estimated ileal protein loss amounts to 25% of the currently recommended minimum protein intake for an adult (0.85 g/kg-day). On a similar basis, in young pigs, the net ileal protein loss is approximately 1.6–2 g/kg-day. This is about 12% of total intestinal protein synthesis, as estimated from the utilization of amino acids (Table 2), and implies that nearly 90% of the secreted protein is digested and recycled. This conclusion is compatible with the limited amount of other published information [3, 43].

The question of intestinal protein secretion and recycling is of particular importance to threonine nutrition. This is because the secretion of mucoproteins, an important factor in the protective mechanisms in the gut, is a significant contributor to overall intestinal protein secretion. Threonine is a site of the extensive o-glycosylation that is characteristic of these proteins [44], and, as a consequence, the core proteins of these molecules are unusually rich in this amino acid [45]. It is probably not coincidental then that the portal availability of dietary threonine is consistently less than that of the other essential amino acids. It is also a frequent observation that, in comparison with the composition of dietary or mixed mucosal protein, the terminal ileal digesta are enriched with threonine. Indeed, as a result of measurements of amino acid losses in ileal effluents in humans receiving protein-free diets. Fuller et al. [39] concluded that the losses of threonine to the large bowel account for approximately 60% of the current estimate of maintenance threonine requirements. Finally, Bertolo et al. [46], in a particularly elegant study, have shown that the threonine requirement of piglets maintained by total parenteral nutrition (a condition in which intestinal growth is greatly reduced and mucin production substantially lowered) is less than half that of animals nourished by the enteral route. It appears therefore, that intestinal protein secretion probably makes a substantial contribution to the maintenance of amino acid needs in general, and to threonine needs in particular.

Amino Acids as Energy Sources in the Gut

In the preceding section, we emphasized that net protein loss from the intestine exerts a nutritionally significant effect on overall amino acid needs. In addition, if amino acids, either taken up from the diet or from the arterial circulation, are metabolized to CO$_2$ and other metabolites, then these pathways will also affect the amino acid requirements of the organism.
The fact that there is mucosal metabolism of dietary amino acids was indicated by early studies showing that the introduction of either glutamate or aspartate into the small intestinal lumen of dogs led to the production of alanine [47]. This observation was not pursued in any detail, until Windmueller and Spaeth [48], in a landmark study, showed that the small intestine of the rat, removed, and metabolized, considerable quantities of arterial glutamine. About 70% of the glutamine carbon was converted to CO2, and they concluded that glutamine was a significant source of energy for the intestinal tissues. This observation has, of course, led to the development of extensive research on the role of glutamine in gut physiology, and there have been many studies that have attempted to show beneficial effects of glutamine on intestinal mass and function.

Regardless of whether these studies have shown functional effects of glutamine, much of this research has assumed, almost axiomatically, that the main role of glutamine in the intestine is as an energy source. While not denying that glutamine is extensively oxidized by the intestine, both in vivo and in vitro [49, 50], we do not believe that its primary role is as a source of energy. It is less well recognized that, in a subsequent study, Windmueller & Spaeth [51] showed that the metabolism of luminal glutamate exceeded that of arterial glutamine. Subsequent measurements of the relative rates of splanchnic glutamate and glutamine metabolism in humans [12, 13], and of intestinal metabolism in piglets [24, 27, 28], have largely confirmed this observation. Moreover, Windmueller and Spaeth [51] also showed that the capacity of the small intestine to metabolize luminal glutamate appeared to be essentially unlimited, while the rate of metabolism of mucosal glutamine appeared relatively fixed. Furthermore, they also found that the introduction of glutamate into the lumen lowered the utilization of arterial glutamine by only about 20%. This suggests to us that, despite the fact that glutamine metabolism must of necessity involve its deamination to glutamate, the metabolic fates of glutamate (taken up from the lumen) and glutamine (taken up from the circulation) are different. This conclusion is compatible with the observations on the differential utilization of luminal and arterial essential amino acids in mucosal protein synthesis [29, 34], and with our studies of mucosal glutathione synthesis [28]. It provides yet another example of metabolic compartmentation in the mucosa in vivo.

Although the studies of Windmueller and Spaeth [26, 48, 51] were notable for their systematic and detailed nature, for very good experimental reasons the majority of these investigations involved the presentation of single substrates to a semi-isolated preparation. Under normal, fed circumstances, however, the mucosa is exposed to a complex mixture of potential substrates. As in vitro data [50] indicate that there are interactions between substrates in the regulation of enterocytic intermediary metabolism, there is no assurance that the quantitative aspects of these earlier investigations, carried out with an isolated jejunal loop preparation, hold in the fed condition in vivo.
Role of the Gut in the Amino Acid Economy of the Host

Table 3. *in vivo* metabolism of different substrates by the portal drained viscera of fed piglets

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Total metabolized</th>
<th>Percent of total metabolism</th>
<th>Unaccounted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/kg/h</td>
<td>percent of input^1^</td>
<td>metabolism to alanine + lactate</td>
</tr>
<tr>
<td>Enteral glucose</td>
<td>220</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>Enteral glutamate</td>
<td>550</td>
<td>94</td>
<td>24</td>
</tr>
<tr>
<td>Arterial glucose</td>
<td>970</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Arterial glutamine</td>
<td>191</td>
<td>20</td>
<td>11</td>
</tr>
</tbody>
</table>

Data are from Stoll *et al.* [27].

^1^ Percent of input refers to the intake of the enteral substrates, and the arterial flux to the portal drained viscera for the arterial substrates.

^2^ Figures in brackets are the percentage of total CO$_2$ production by the portal drained viscera that was derived from each substrate.

We [27] have investigated this question in portal catheterized, unanesthetized, and fully fed piglets [52] receiving, either enterally or intravenously, [U-13C]glutamate, glutamine, or glucose. The results are summarized in Table 3. Although, in a qualitative sense, the results confirmed previous findings [26, 48, 51], they also revealed some other interesting aspects of mucosal intermediary metabolism, some of which were the subject of specific comment by Windmueller and Spaeth [26].

First, there was clear compartmentation of glucose metabolism. Thus the first-pass intestinal metabolism of dietary glucose was very low. Furthermore while virtually all the enteral glucose utilized by the mucosa was catabolized, 70% of this was directed to the production of alanine and lactate. On the other hand, the portal drained viscera utilized 25% of the whole body glucose flux, but only a third of this was recovered in CO$_2$ and three-carbon metabolites in the portal blood. Thus arterial glucose served primarily as a biosynthetic precursor within the viscera. As young pigs deposit very little abdominal fat, it is tempting to speculate that nucleic acid and secretory glycoprotein synthesis are important factors in the utilization of arterial glucose by the mucosa.

Some aspects of the amino acid data were also particularly interesting. First, although the proportion of the enteral glutamate oxidized to CO$_2$ (57%) was less than the proportion of arterial glutamine oxidized (72%), glutamate catabolism contributed 2.5-fold more CO$_2$ than glutamine metabolism. In addition, the production of newly synthesized alanine (which accounted for more than half the portal alanine balance) did not account for all the nitrogen generated from the catabolism of glutamate, aspartate, and glutamine, and there was a substantial (fivefold) increase in the production of ammonia by the gut as soon as
Role of the Gut in the Amino Acid Economy of the Host

Table 4. Preliminary data on leucine and lysine oxidation by the portal drained viscera of milk-fed piglets

<table>
<thead>
<tr>
<th></th>
<th>Leucine</th>
<th>Lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake, µmol/kg/h</td>
<td>444</td>
<td>422</td>
</tr>
<tr>
<td>Oxidation by extravisceral tissues, µmol/kg/h</td>
<td>148</td>
<td>45</td>
</tr>
<tr>
<td>1st-pass intestinal metabolism, µmol/kg/h</td>
<td>138</td>
<td>135</td>
</tr>
<tr>
<td>Intestinal oxidation, µmol/kg/h</td>
<td>44</td>
<td>23</td>
</tr>
<tr>
<td>Percent of first-pass metabolism</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>Percent of whole body oxidation</td>
<td>23</td>
<td>34</td>
</tr>
</tbody>
</table>

1 Unpublished data from Burin et al.
2 Unpublished data from van Goudoever et al.
3 Calculated from estimated rates of protein deposition and portal leucine balance.

the animals were fed [22]. Indeed, ammonia production accounted for 20% of total protein nitrogen intake, and considerably exceeded the measured rate of glutamine deamidation. Moreover, the fact that the combined exit of metabolic nitrogen in alanine, arginine, and ammonia (1250 µmol N/kg-h) exceeded the total catabolism of glutamate, aspartate, and glutamine (980 µmol N/kg-h) suggests that other amino acids were being catabolized by the intestine. The excess production of ammonia was also noteworthy in another respect, because it implies that there is active glutamate dehydrogenase activity in the mucosa [53]. This in its turn provides a mechanism whereby the nitrogen derived from the catabolism of amino acids, other than aspartate, glutamate, and glutamine, can be accommodated within mucosal intermediary metabolism.

We are currently investigating whether other amino acids are catabolized by the intestinal tissues. Preliminary results (Table 4) show that, as found in other studies [15, 16], there is significant leucine catabolism. Critically though, we have found that enteral lysine (which was the first-limiting amino acid in the diet) is also catabolized by the mucosa. Indeed, in these rapidly growing animals, leucine and lysine catabolism by the portal drained viscera accounted for 23 and 34%, respectively, of their total body oxidation. Furthermore, lysine metabolism is apparently compartmentalized because, although the intestinal tissues metabolized lysine from both luminal and arterial sources, only the luminal source was oxidized.

However, regardless of the eventual results of our studies of essential amino acid catabolism by the gut, the fact remains that amino acids, especially nonessential amino acids, appear to be the major if not the preferred source of energy for the intestinal mucosa. This has clear implications for nonessential amino acid biosynthesis and for both systemic glucose and branched chain amino acid metabolism.
Role of the Gut in the Amino Acid Economy of the Host

Role of Amino Acids in Nonprotein Pathways of Biosynthesis in the Mucosa

Apart from its substantial impact on whole body protein turnover and energy expenditure, the intestine is also a site of various other important biosynthetic pathways, several of which use nonessential amino acids as precursors (Fig. 3). First, despite the high rate of cellular proliferation in the mucosa, it appears that mucosal nucleic acid synthesis utilizes diminishingly low quantities of preformed pyrimidines and purines [54], at least of dietary origin. Thus the synthesis of mucosal nucleic acid precursors relies on de novo synthesis of their nucleotide bases [55]. It follows from this that glycine, glutamine, and aspartate may play key roles in the support, and perhaps the regulation, of mucosal nucleic acid synthesis. Certainly, the incorporation of intact glycine [56] and aspartate [55] molecules into mucosal nucleic acids is readily demonstrable.

The gut is also an important site for the synthesis of amino acids. We have already highlighted the substantial synthesis of alanine that occurs in the intestine, but it is also clear that the mucosa is a critical contributor to the body’s supply of proline and arginine, amino acids that are derived from glutamate and glutamine metabolism. In rats, it has been known for many years that the synthesis of arginine involves the cooperative activities of the mucosa, as a source of citrulline [57], and the kidney, which utilizes this citrulline for arginine synthesis [58]. The physiological and nutritional importance of this pathway is strikingly illustrated by the fact that massive gut resection renders arginine a folly essential amino acid [59]. Moreover, inhibition of ornithine aminotransferase, a key enzyme in mucosal citrulline synthesis, lowers circulating arginine levels [60]. It is of particular interest that in young pigs the intestinal mucosa appears to be able to carry out the net synthesis of arginine [61, 62], and isotopic evidence [27] shows that this is released to the portal circulation. This
Role of the Gut in the Amino Acid Economy of the Host

is of particular importance, because milk is relatively deficient in arginine [63], so that arginine biosynthesis is of specific importance to the young mammal. Indeed, it appears that arginine synthesis may continue into later periods of development, but because of the appearance of mucosal arginase as the animals approach weaning [64], arginine release from the gut (as opposed to citrulline release) becomes negligible. It is also of nutritional and clinical significance that, once again, the biosynthesis of both arginine and proline appears to be compartmentalized. Thus enteral glutamate [65] and proline [66] are apparently obligatory precursors for mucosal proline [65] and arginine [66] biosynthesis, respectively. This would be entirely in keeping with the observation that individuals maintained by parenteral nutrition are at severe risk of arginine deficiency and associated hyperammonemia, even though arterial glutamine, a potential precursor for these pathways, and intestinal glutamine uptake do not appear to be compromised during total parenteral nutrition.

The metabolic compartmentation, which increasingly appears to be a feature of mucosal metabolism, extends to other biosynthetic activities. The gut is exposed to many potential toxins of dietary or bacterial origin. Glutathione, the mucosal concentration and synthesis of which is very high (200–500% day) [28, 67], plays a critical role in these protective mechanisms. Mucosal glutathione synthesis can be compromised by restricting the protein intake of the animal [66]. It appears from studies with [U-13C]glutamate and glycine [28] that mucosal glutathione derives specifically from enteral (dietary) rather than systemic precursor amino acids. Finally, it seems likely to us that an important aspect of glutamine function in the mucosa is the support of the amino sugar synthesis that is necessary for the continuing synthesis and secretion of the secretory mucins.

Conclusion

Although interest in intestinal metabolism has a history extending back for at least 50 years, systematic in vivo investigations on the impact of gut metabolism on the host are of relatively recent provenance. The mucosa, in particular, consumes disproportionate quantities of amino acids for protein synthesis and have a commensurately high rate of energy expenditure. Thus, in the most general sense, the intestinal tissues are major factors in the regulation of energy expenditure [68] and protein turnover. In regard to protein synthesis, various important questions remain unanswered. Chief among these is a lack of quantitative information on the in vivo rate of synthesis, and the degree of recycling of the protein secretions. This is, in our view, critical information, for no other reason than many intestinal diseases appear to be accompanied by increased secretory outflow which, in turn, will have an impact on the amino acid utilization [69] and hence on the growth of the organism.
Role of the Gut in the Amino Acid Economy of the Host

Another clear feature of mucosal metabolism that remains to be understood at the mechanistic level is the high degree of metabolic compartmentation. This compartmentation has the important consequence that the source of an amino acid (luminal or systemic) can have a crucial bearing on the pathways that it follows in the mucosal cells. Furthermore, although it now appears that amino acids are critical energy sources for the intestinal mucosa, important questions about the regulation of these pathways, and in particular whether they are subject to nutritional regulation, remain uninvestigated. Despite these substantial areas of current ignorance, it is now very clear that the intestine is not ‘merely’ an organ of nutrient assimilation, but an important point of host defense that uses substantial quantities of amino acids to maintain functions of critical physiological importance.

Acknowledgements

This work is a publication of the USDA/ARS Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children’s Hospital, Houston, Tex., USA. The work was supported in part by federal funds from the US Department of Agriculture Agricultural Research Service, Cooperative Agreement No. 58-6258-6001, by the National Institutes of Health (ROI HD33920 and ROI-HD 35679) and by the International Glutamate Technical Committee. J.B. van Goudoever was supported by the Sophia Foundation of Scientific Research, Nutricia Research Foundation, and the Royal Netherlands Academy of Arts and Sciences (Ter Meulen Fund). The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government. We thank L. Loddeke for her careful editing of this paper.

References

Role of the Gut in the Amino Acid Economy of the Host

Role of the Gut in the Amino Acid Economy of the Host


47. Neame KB, Wiseman G. The transamination of glutamic and aspartic acids during absorption by the small intestine of the dog in vivo. *J Physiol (Lond)* 1957; 135: 442–50.


Role of the Gut in the Amino Acid Economy of the Host


65. Murphy JM, Murch SJ, Ball RO. Proline is synthesized from glutamate during intragastric infusion but not during intravenous infusion in neonatal piglets. *J Nutr* 1996; 126: 878–86.


Discussion

**Dr. Breuillé:** One thing that seems particularly interesting is that you found that glutamate first-pass extraction was very high and at the same time you demonstrate that the first utilization of glutamate was for glutathione synthesis in the intestine. So my question is, have you any idea about first-pass extraction of the other amino acids necessary for glutathione synthesis, for example glycine and cysteine?

**Dr. Reeds:** We have rarely if ever found a positive portal cysteine balance in our piglets, which implies that 100% of the dietary cysteine is being used in first pass. First-pass splanchnic utilization of cysteine in the human is much higher than in many other species, and it is very much channeled to glutathione synthesis. We’ve done some cysteine depletion studies and it has a remarkably small effect on glutathione status in the mucosa, though it has interesting effects on secretion. It looks as though the gut can either resynthesize cysteine from methionine readily or that it switches over to cysteine utilization from the artery.
Dr. Lundholm: You emphasized that lysine taken up from the luminal side was oxidized under certain conditions, while lysine taken up simultaneously from the arterial side was not oxidized at all. Do you then propose that there is a division among the different intestinal cells, such that some cells completely oxidize lysine, while others are not exposed to amino acids for lysine oxidization?

Dr. Reeds: That’s an obvious conclusion, but there is as yet no technique to readily fractionate cells from the small intestine. If one is interested in the functional and nutritional consequences, one needs to study the mucosa as an entity, like studying the liver as an entity, but one is still left with mechanistic worries. Is it the crypt cells and the lymphocytes that are carrying out this compartmentation? How much of what we observe is bacterial metabolism, for example? We don’t really know about the contribution of the resident flora in the small intestine to overall intestinal metabolism. I wish I could answer that question.

Dr. Lundholm: It sounds very unphysiological to me that the body should divide some cells for lysine oxidation and some only for lysine incorporation in protein synthesis. I prefer a model that proposes incomplete mixing in the compartment. We know that the models using tracer kinetics are not valid, so I think we have a very fundamental scientific problem here that we must resolve.

Dr. Reeds: To some extent the problem of compartmentation is reasonably well solved if you can get tracers into the two compartments and identify them individually, because one can study it at a regulatory level. Frankly, I think that it’s going to be impossible, or at least very difficult, to study the compartmentation on a cell type by cell type basis. Nonetheless I think that what you have said is by far the most likely explanation for a number of these phenomena.

Dr. Young: Surely you could get at it by measuring the activity of lysine α-ketoglutarate reductase activity in various cell types, and at least get at the possible compartmentation of lysine oxidation in that way?

Dr. Reeds: Attempts have been made to measure that enzyme in the mucosa [1] and they found no activity. The only problem with that is the relevance of in vitro enzyme activity. Does the result reflect the activity in vivo as well? And when you do the sums, the mucosa is exposed to such high topical concentrations of amino acids that you don’t need very much enzyme activity to account for these phenomena. I’ll give you an example. The mucosa does have glutamate dehydrogenase, and we get substantial ammonia production from the gut, which we could not account for by glutamine deamination. It’s far too much for that. In fact, the glutamate dehydrogenase is extremely low in comparison with the liver, but it’s more than enough to cope with glutamate metabolism of 600 µmol/kg-h.

Dr. Jackson: Could I ask you to be slightly clearer about how you carry out your studies, because clearly there will be longitudinal changes in the gastrointestinal tract as one goes from the proximal to the distal end, so one might reasonably expect that the impact of a dietary or luminal component would be greater proximally and less distally. Thus you might get compartmentation simply in terms of that gradient along the gut.

Dr. Reeds: Our measurements include all the gut save the rectum. We have the catheter almost in the liver. The pig has a rather short common portal vein and in fact it’s beyond the entry of the gastrosplenic vein as well.

Dr. Jackson: My second question relates to what it is you call ‘portal biliary drainage’ and where you are taking the samples from, and which parts of the small intestine/bowel you are including in that measurement. Another important variable is the relative contribution of enterohepatic cycles through gut secretions to the variables you are measuring, and what part hepatic function plays in this. That must be an important variable in terms
Role of the Gut in the Amino Acid Economy of the Host

of glutathione cycling. Finally, maybe you could comment on the role of diurnal variation in terms of the relative proportion of gastrointestinal protein turnover to total body protein turnover. As you know, in humans we find a very sharp diurnal variability with continuous food intake around about midnight, and we feel this is probably related to switches either in the gut or the liver. When you make the extrapolations to 50% of whole body protein turnover, is that a reasonable extrapolation, or is it 50% of total body protein turnover at the time you were studying it on the day and under those conditions?

Dr. Reeds: I accept entirely the point that we are studying gut metabolism under very specific physiological circumstances, and we study it under those physiological circumstances because we are interested in intestinal metabolism round about the weaning transition and because this is also a stage of development where the gut is making its largest contribution to body weight. We have also noted that in animals on continuous feeding there is a slow periodicity to the portal balance, the degree of metabolism, and indeed the utilization of arterial amino acids, so there is a regulatory loop operating which none of us can identify at this stage. There are intriguing data now emerging in relation to ileal signals regulating proximal activity, peptides such as GLP2, for example [2].

In relation to glutathione, I think one of the most remarkable things that came out of our glutathione experiments was the fact that the isotopic enrichment of the glutathione in the lumen was almost exactly the same as the enrichment of the glutathione in the mucosa, so there’s a very high secretory component to that glutathione ‘turnover’. That means that, because we were using tracers (glutamate and cysteine) that aren’t absorbed very efficiently, the liver GSH would have had extremely low labeling. My suspicion is that the GSH that is found in bile is largely conjugated already, and that won’t be picked up in the method that we’re using, which is specifically designed to measure reduced free glutathione.

Finally, in relation to protein metabolism the real key variable to my mind is to be able to quantify the secretions, which is what we’re planning to do next partly because of the carbohydrate implications of that, and then to quantify the recycling because it’s the ability to recycle secretions that’s the crucial aspect of the impact of gut on essential amino acid requirements.

Dr. Young: All of these quantitative estimates, of course, are based on your piglet model. Ultimately, we’re interested in the human. The piglet’s dynamic state of protein metabolism is tremendous relative to, let’s say, the adult human, so I wonder to what extent you feel confident that the quantitative relations that you so beautifully discussed with respect to the piglet apply to the adult human?

Dr. Reeds: I think they do. All the data of which I’m aware show the close relation between level of intake and gut metabolism. I accept that the piglet is growing, but the fundamental difference is the voluntary appetite of the two species. Now if you measure secretory outflow in the human – as has been done by giving labeled diets and then looking at endogenous unlabeled and labeled nitrogen [3] – and then compare it with the pig, you find that, though there is a substantial difference in the quantities, they are almost exactly the same per unit of dry matter intake – that is, about 25 g protein/kg dry matter. So the gut, being so mass driven if you like, is following faithfully the appetite of that individual species. One of the reasons why I tried to put so much interspecies data together in the paper and presentation was to convince you that these aspects of animal modeling can be used in humans as well, providing you’re aware that you’re dealing with a species, the human being, that lives close to maintenance.

Dr. Boza: In inflammatory bowel disease there is said to be impaired synthesis of mucoproteins or at least altered secretion of mucoproteins [4]. Do you think this could be modulated nutritionally, for instance by threonine and glutamine supplementation?
Dr. Reeds: There’s a short answer to that: with enormous difficulty! It is crucially important to try and get a handle on mucin synthesis. I didn’t have time to point out that although the gut is taking up a lot of arterial glucose only about one third of that is catabolized; in other words two thirds is disappearing into some biosynthetic pathway. As pigs don’t synthesize abdominal storage fat to any great extent, there’s only really one pathway that could be consuming such a large quantity of glucose and that’s carbohydrate transformations. I feel fairly certain that the changes in leucine dynamics in response to parasitic infection were due to the enormous secretory response, because that is the gut’s common response to all forms of stress – it doesn’t matter whether it’s a dietary lectin or a bacterial toxin, it immediately induces mucin synthesis. There’s some intriguing new evidence emerging that there may even be induction of the development of goblet cells during TPN [5], so there is a possibility that separate differentiation pathways are regulated by these stimuli.

Dr. Fürst: I would like to touch on possible species differences. If I remember correctly, there was a paper in around 1980 in the Journal of Biological Chemistry which showed about 52% glutamine oxidation in rats [6]. I believe this was later confirmed in dogs [7]. This is an enormous difference from the value you claim for piglets.

Dr. Reeds: I hate to be critical of papers which had a substantial influence on clinical nutrition, but in that last paper they attempted to study fed animals – in other words they infused amino acids into an isolated loop, but the amino acids were also in a bicarbonate buffer, so they couldn’t actually measure CO2 production because it was diluted by bicarbonate exchange. Now we happen to know what the relation between intake and expenditure is, and if we assume that this relation is essentially the same in the rat and the pig, we find that the contribution of glutamine falls quite markedly. Therefore I think those were overestimates of glutamine oxidation. There is still a very substantial amount of glutamine oxidation going on, but in the fed state it accounts for a relatively small proportion of CO2. In the fasted state it may be a different story.

Dr. Fürst: My second question concerns glutathione synthesis and the glutamine/glutamate complex. We have learned in our textbooks, and also seen from experimental evidence, that highly charged molecules, like glutamate, are poorly transported across the cell membrane, so they might thus be very poor precursors if given exogenously. This was beautifully shown in a paper by Mittendorfer et al. [8]. So my question is, how can this information be compatible with your data? This is a practical question related to what to give in enteral nutrition to obtain glutathione synthesis.

Dr. Reeds: It is clear – because we’ve made the measurements – that glutamate is being absorbed from the lumen, thus it’s not a removal problem. Indeed glutamate has to be absorbed: 20% of the intake of the milk-fed infant is glutamine and glutamate, and I presume, because they’re growing, that they are absorbing them – they’ve got a dreadful biosynthetic problem if they’re not! The puzzle is that if you isolate enterocytes they are remarkably reluctant to take up glutamate, and the data obtained from enterocytes hold true throughout the literature: glutamate is taken up but to a very modest extent. In other words, either the glutamate is being presented to the gut in a different form, and it’s conceivable that dipeptides are much more important in that respect, or there’s something that we really do not know about glutamate transport in vivo.

Dr. Fürst: My third question is a very short technical one. How did you measure the true glutamate content in the diet? If you use your amino acid analyzer to measure, you will get a sum of glutamine and glutamate.

Dr. Reeds: We got the manufacturers to tell us which proteins they had added. We then went to the gene base and obtained the answer from the coding sequences of the proteins. If you do the analysis by hydrolysis, 21% of the dietary protein is glutamate, of which 40% is glutamine.
Role of the Gut in the Amino Acid Economy of the Host

Dr. Beaufrère: We seem to consider that splanchnic extraction of dietary amino acids is a sort of global index of splanchnic protein metabolism. You gave a figure in humans of 30% for leucine, but as you know there is wide variation in the literature and I think that published values run from around 5 to 35%. It is quite striking that different research groups always find the same total leucine flux, and the variability of leucine flux is probably not more than 20–40% among groups. This is very different for splanchnic leucine extraction. My question is, do you have the same problem in piglets, and second, if this variability is real and not a propagation of error due to the model, do you see any physiological explanation for it?

Dr. Reeds: As Dr. Young pointed out, one needs to look very carefully at what the protein intakes were in those studies. Those were fed splanchnic extractions. I’m not quite sure what the splanchnic extraction of an enteral amino acid means in the fasted state, but this gives me an opportunity to introduce you to something really remarkable. If you put piglets on a diet that maintains body protein mass – that is, you put them down to maintenance – portal balance is virtually zero, but gut lysine utilization continues at the same rate as when the piglets were receiving three times the protein intake. So I think that gut metabolism may represent a fixed cost: you could almost argue that the gut is a selfish organ. I would argue that you have to protect that particular surface at all costs.

Dr. Beaufrère: That seems to imply that the variability would have to be explained by the liver?

Dr. Reeds: No. I think some of the variability is undoubtedly analytic; it always is. But some of it occurs because not everyone feeds quite the same quantities of the amino acids they were studying. A good example is the phenylalanine data from Tessari’s group and the data from your old group. There were differences in protein intake between those two studies. If the lysine data that we are getting prove to be correct, this implies that first-pass splanchnic extraction will involve a higher proportion of the tracer as the intake of that amino acid or protein goes down, and it’ll end up as the same number of micromoles.

Dr. Millward: I’d like to go back to the issue of some of the mechanisms involved. I found your talk fascinating and very puzzling. For example you say that the isolated enterocyte doesn’t take up glutamate?

Dr. Reeds: Most cells are very reluctant to take up glutamate.

Dr. Millward: Well, in the context of what Dr. Lundholm asked, the gut is made up of enterocytes and other cell types, and a large fraction of these other cell types are immune cells. I don’t know much about the anatomic location of these and where they sit in relation to systemic blood flow and the luminal supply, but is it possible that these cells participate in amino acid uptake into the host? I mean, can we think of them as having a luminal side and a systemic side?

Dr. Reeds: To some extent, yes, because they are sitting within the lamina propria, and between enterocytes into which the dietary amino acids are emerging. It’s conceivable that it’s the lymphocytes that are carrying out all these rather intriguing biosynthetic activities. At this point it’s very difficult to see how one could sort out that problem. This is of enormous concern to us because of its mechanistic implications.

Dr. Millward: Could you use the newer techniques of cell sorting to work some of this out?

Dr. Reeds: I suppose you could, and then you’d have to go and look for the enzyme or the message, and hope that you’d got the right enzyme! If the gut is healthy there is actually a remarkably small number of macrophages and lymphocytes. They account for no more than 2–3% of the total cells if you do quantitative histochemistry. Their numbers increase a lot in total parenteral nutrition, as do goblet cell numbers in animals maintained
Role of the Gut in the Amino Acid Economy of the Host

by TPN. Thus I think it’s unlikely that they’re responsible for all this metabolism, but that’s just a hope and a prayer at this stage. I’m more concerned about bacteria than anything else in the interpretation of these data.

Dr. Young: You mentioned that one fate of glutamate in the mucosa is with respect to arginine synthesis. As you well know, it was shown a number of years ago that there is a profound developmental change in the capacity of the gut for arginine synthesis [9]. You are dealing with a young piglet. Do you have any idea of whether or not there is developmental downregulation of arginine synthesis in the pig?

Dr. Reeds: What seems to have happened in the pig is that just after the age at which we’re studying them, arginase activity rises, so that they run a complete urea cycle. However, Deutz has direct evidence for net arginine synthesis within the gut in somewhat older pigs [personal communication, ESPEN poster], and I think that the human gut can synthesize some arginine. The amount will depend upon the activity of arginase within the system, but happily that still releases ornithine, which could in theory go to arginine elsewhere. Did I take your slide on arginine synthesis to mean that when somebody is severely uremic they are still able to synthesize arginine?

Dr. Young: Yes, that’s correct, so the implication is that arginine synthesis is occurring somewhere other than in the kidney.

Dr. Reeds: Or that the inability to make arginine in the kidney is a very late event in uremia.

Dr. Young: Well, multicatheterized dog model studies suggest that arginine synthesis in the adult dog occurs also ex-kidney, presumably in the splanchnic region and possibly in the intestine [10].

Dr. Déchelotte: The gut is a complex organ with a large amount of smooth muscle and cyclic activity. Do you have any idea whether the cyclic motor function influences gut metabolic needs and the utilization of any particular amino acids?

Dr. Reeds: I don’t know, but I presume it does. The metabolism of smooth muscle cells and enterocytes, and the enterocytes win out very substantially in terms of oxygen consumption. I wish we could look more into motility, because we’re coming increasingly to the conclusion that gastric emptying and motility are of crucial importance in some of the diurnal variations we’re starting to pick up.

Dr. Lundholm: I would like to add to Dr. Millward’s question about model validity. We shouldn’t forget about the endothelial cells in the gut. On a per weight basis those cells are extremely metabolically active. We have done some experiments on the expression of nitric oxide and other regulatory peptides along the intestinal tract and it varies enormously from north to south. So we ought not to talk as though the gut was a homogeneous organ; we must specify which part of the gut we are discussing. I would like to ask you about the contribution of the large bowel to gut metabolism in your experiments. As a surgeon, I think it would be very difficult to dissect out the contribution of the large bowel to the intestine as a whole unless you do vascular preparations on your piglets.

Dr. Reeds: We hadn’t thought about this at the time we set up these experiments, but by complete good luck, these pigs are just before the stage in their development when there is a large increase in large intestinal mass. If you look at the relations between the utilization of arterial amino acids and gut mass in cross-species studies, the results fit really quite well with the contribution of the large bowel to overall gut mass. The colonic mucosa appears to have a relatively low rate of protein synthesis – about one third of the small intestinal mucosa. I wish there was some way of separating the large and the small bowel by appropriate catheterization, if for no other reason than to demonstrate that the ammonia production we find is predominantly in the small rather than the large intestine.
Role of the Gut in the Amino Acid Economy of the Host

References