Homeostasis of the Host

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ABSTRACT Among the reasons suggested for the discrepancy between N balance and tracer-derived indispensable amino acid (IAA) requirement estimates is the possibility that the metabolic requirement is met not only by the diet but also by IAA synthesized de novo by the gastrointestinal microflora, which are then absorbed. It is therefore crucial to better understand and quantify the microbial biosynthesis of amino acids in the human gastrointestinal tract and its potential role in providing IAA to meet human amino acid requirement. Here, the available evidence on the contribution of microbial amino acids to the host’s amino acid homeostasis, applying the 15N labeling paradigm, is summarized. Between 1 and 20% of circulating plasma lysine, urinary lysine and body protein lysine of the host, respectively, is derived from intestinal microbial sources and corresponds to a gross microbial lysine contribution of 11–68 mg·kg−1·d−1 in adult humans with an adequate protein intake when fecal or ileal microbial lysine enrichment is used as precursor. Factors affecting estimates of net microbial IAA contribution are discussed. It appears that the small intestine is responsible for a large part of microbial lysine uptake, although some absorption from the large intestine cannot be excluded. Nonoxidative lysine losses from the human gastrointestinal tract, which were found to be between 3.9 to 8.5 mg·kg−1·d−1, are necessary to estimate the net contribution of microbial IAA. It is reasonable to assume that microbial amino acid synthesis in the human gastrointestinal tract utilizes a mixture of various nitrogen sources, i.e., endogenous amino acids, urea and ammonia. Microbes in the small intestine may rely more on endogenous amino acids. Deprivation of nutrients, the intake of certain dietary nonstarch oligosaccharides, lipids, as well as protein intake level and source and level of consumption of certain amino acids can affect the composition and metabolic activity of the intestinal microflora and thus its fermentation products potentially available to the host. In conclusion, with the use of the 15N labeling paradigm, a significant contribution of microbial lysine to the host lysine homeostasis is found. However, to assess the net contribution of microbial IAA and its importance in defining the adult IAA requirement, this is not the ultimately successful experimental strategy because the interpretation of results is complicated by the nitrogen recycling in the gut, the uncertainty of the precursor pool of absorption and the limited data on nonoxidative IAA losses from the human gastrointestinal tract. J. Nutr. 130: 1857S—1864S, 2000.

KEY WORDS: • intestinal bacteria • amino acid synthesis • tracer balance • large intestine • amino acid requirement

The human intestinal tract is colonized by >400 species of bacteria (Gordon et al. 1997) from the small to the large intestine, but they are not uniformly distributed in either number, species or metabolic activity (Table 1) (Autenrieth 1998, Goldin 1990, Hovgaard and Brondsted 1996). Thus, for persons living in Europe and North America, microbial counts increase along the intestinal tract, with a bacterial concentra-

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been shown that microbial activity is responsive to metabolic and pathogen challenges (Hendrickson et al. 1999, Mack et al. 1999, Spitz et al. 1994) and dietary factors (Alverdy and Stern 1998, Kim et al. 1998, Macfarlane and Cummings 1999) but also can communicate with the host cells to cause them to fulfill their needs as shown for Bacteroides thetaiotaomicron (Gordon et al. 1997, Hooper et al. 1998). The rhizobium-legume symbionts serve as an example for nonpathogenic interactions in which the secretion of soluble factors by the bacteria symbiont influences development and differentiation of the host.

Among the numerous biochemical reactions of which microorganisms are capable (Bengmark 1998, Goldin 1990) is the ability of amino acid production from nonspecific nitrogen sources driven by energy generated from dietary and endogenous fermentable carbohydrates (Matteuzzi et al. 1978, Reitzer and Magasanik 1996, Sauer et al. 1975). Various human and animal studies show that the administration of nonspecific 15N (e.g., urea, ammonium chloride) can be used to label microbial lysine and threonine, which is subsequently termed the 15N labeling paradigm.” The appearance of 15N-labeled lysine and threonine in body fluids or proteins presumably indicates their absorption from microbial sources (Torrallardona et al. 1996a) because these amino acids are not transaminated by mammalian tissues.

Among the reasons suggested for the discrepancy between N balance and tracer-derived amino acid requirement estimates is the possibility that the metabolic requirement, i.e., the irreversible loss of indispensable amino acids (IAA) (of which oxidation is the major component), is not met by the diet but also by amino acids synthesized de novo by the gastrointestinal microflora, which are then absorbed. It is therefore crucial to better understand and quantify the microbial biosynthesis of amino acids in the human gastrointestinal tract and its potential role in providing IAA to meet human amino acid requirement (Fuller et al. 1997, Hooper et al. 1998) discuss to determine the adult requirement of IAA.

Estimation of microbial lysine contribution to lysine homeostasis of the host using 15N labeling paradigm

It was observed earlier in uremic patients and in subjects consuming low protein diets (Giordano et al. 1968, Tanaka et al. 1980) that microbial lysine can be incorporated into host body protein, but no attempt had been made to quantify this contribution. Utilization of microbially derived IAA has been confirmed in pigs and rats fed protein-free or low protein diets (Torrallardona et al. 1994 and 1996a), whereas it was reported that absorption of microbial lysine in the rat was exclusively due to coprophagy (Torrallardona et al. 1996b). In a study in protein-energy–malfourished infants, the appearance of urinary 15N lysine after the oral intake of 15N urea was observed (Yeboah et al. 1996). In recent studies in adult humans on nitrogen (protein)-adequate diets, we observed a significant contribution of microbially derived lysine and threonine to the free plasma lysine and threonine pool (Metges et al. 1999a and 1999b).

In the following, the experimental design used and the main outcomes of our studies are summarized, and results are compared with similar studies in animals and men. Healthy young adults and a group of otherwise healthy subjects with ileostomies were adapted to an adequate diet based on crystalline amino acid mixture for four days (Metges et al. 1999a). On two different occasions and after taking appropriate baseline samples between days 5 and 11, isonitrogenous amounts of 15NH4Cl or 15N2 urea, respectively, were added daily to the diet of the normal subjects to label microbially synthesized amino acids. The ileostomy subjects were studied with 15NH4Cl only. The comparison between 15NH4Cl and 15N2 urea was made because a pilot study in minipigs suggested a different metabolic and microbial fate of those two sources of nonspecific nitrogen (Metges et al. 1996). The study of the two groups of subjects, one with an intact gastrointestinal tract and one without a large intestine (ileostomates), enabled us to compare estimates of microbial lysine and threonine contribution by using ileal and fecal microbial protein as putative precursor for lysine and threonine absorption. The use of gas chromatography–combustion isotope ratio mass spectrometry allowed us to measure 15N enrichment of circulating free plasma amino acids, which would have not been possible using preparative ion exchange (Torrallardona et al. 1996a, Yeboah et al. 1996). The fractional appearance of microbial lysine (or threonine) in the circulating plasma was calculated as the ratio of plasma free 15N lysine (or 15N threonine) to the presumable precursor, i.e., fecal or ileal microbial protein-bound lysine (or threonine), respectively (Table 2). In an attempt to quantify the microbial lysine and threonine contribution to the host homeostasis, we multiplied this ratio by the plasma lysine and threonine turnover, respectively, measured via intravenous 13C lysine infusion in the same subjects or taken from the literature (Zhao et al. 1986) (Table 2). Microbial threonine

### TABLE 1
Microbial counts along the human gastrointestinal tract

<table>
<thead>
<tr>
<th>Location</th>
<th>Length</th>
<th>Transit</th>
<th>pH</th>
<th>Bacterial counts</th>
<th>Abundant species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity</td>
<td>0.6</td>
<td>1–60 s</td>
<td>1–5</td>
<td>0–104</td>
<td>Lactobacilli</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.2</td>
<td>1–5</td>
<td>5–7</td>
<td>0–103</td>
<td>Lactobacilli, Bacteroides</td>
</tr>
<tr>
<td>Duodenum</td>
<td>1.5</td>
<td>2–6</td>
<td>106–9</td>
<td>Enterobacteria, Bacteroides</td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>2</td>
<td>1–5</td>
<td>6–7,5</td>
<td>Bifidobacteria, Enterococci</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>1.8</td>
<td>8–70</td>
<td>5.5–7</td>
<td>Enterococci, Enterobacteria, Clostridia, Bacteroides</td>
<td></td>
</tr>
</tbody>
</table>

1 Adapted from Autenrieth 1998 and Hovgaard and Brondsted 1996.
Site and precursor pool of microbial amino acid absorption

The scientific literature has been largely concerned with the impact of colonic fermentation products affected by the intake of prebiotics and probiotics (Macfarlane and Cummings, 1999) in regard to colon cancer protection and systemic immunity. A possible role of the ileal microflora for host amino acid nutriture has not been considered so far. In contrast to the available evidence that quantitative important amino acid absorption occurs only in the small intestine, the possibility of amino acid absorption from the large intestine should not generally be excluded for reasons indicated later. In addition, it is still an open question in which form microbial amino acids are absorbed (i.e., free amino acids or peptides). Because use of the $^{15}$N labeling paradigm requires knowledge

### TABLE 2
Appearance of various microbial indispensable and nonindispensable amino acids in the plasma pool measured after oral administration of $^{15}$NH$_4$Cl and $^{15}$N$_2$ urea$^1$

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>$^{15}$NH$_4$Cl</th>
<th>$^{15}$N$_2$ urea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Lysine</td>
<td>21</td>
<td>67.9</td>
</tr>
<tr>
<td>Threonine</td>
<td>17</td>
<td>44.7</td>
</tr>
<tr>
<td>Valine</td>
<td>80</td>
<td>88</td>
</tr>
<tr>
<td>Histidine</td>
<td>52</td>
<td>49</td>
</tr>
<tr>
<td>Proline</td>
<td>41</td>
<td>73</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>72</td>
<td>138</td>
</tr>
<tr>
<td>Alanine</td>
<td>63</td>
<td>111</td>
</tr>
<tr>
<td>Glycine</td>
<td>93</td>
<td>182</td>
</tr>
</tbody>
</table>

$^1$ Summarized from Metges et al. 1999a, 1999b.  
$^2$ Ileal effluent of ileostomy subjects.  
$^3$ Normal subjects.  
$^4$ Threonine plasma flux taken from Zhao et al. 1986.

and lysine contribution ranged from 8 to 17% and from 5 to 21%, respectively. These estimates correspond to a microbial contribution of lysine and threonine to the plasma flux ranging from 11.7 to 67.9 and from 21 to 44.7 mg·kg$^{-1}·d^{-1}$ in normal adults, respectively.

For comparative purposes, Table 3 presents a summary of the available data on microbial lysine appearance in the host body protein or amino acid pools in rats, pigs and humans. In applying the $^{15}$N labeling paradigm, between 1 and 20% of circulating plasma lysine, urinary lysine and body protein lysine of the host is derived from intestinal microbial sources (Table 3). There is a trend toward higher numbers when the ileal microbial protein was used as precursor, whereas it appears that the calculated contribution to body protein lysine is lower.

### TABLE 3
Contribution of microbial lysine to host plasma free, urinary and body protein lysine pool: Comparison of data from rats, pigs and human subjects measured via oral administration of nonspecific $^{15}$N

<table>
<thead>
<tr>
<th>Species</th>
<th>Dietary intake</th>
<th>$^{15}$N tracer</th>
<th>Microbial lysine</th>
<th>Host lysine</th>
<th>Contribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal adult2</td>
<td>11/186$^2$</td>
<td>$^{15}$NH$_4$Cl</td>
<td>Fecal</td>
<td>Plasma</td>
<td>7.53</td>
<td>Metges et al. 1999a</td>
</tr>
<tr>
<td>Ileostomy patient</td>
<td>11/134$^2$</td>
<td>$^{15}$NH$_4$Cl</td>
<td>Fecal</td>
<td>Plasma</td>
<td>4.6$^3$</td>
<td>Yeboah et al. 1996</td>
</tr>
<tr>
<td>Malnourished infants</td>
<td>0.5–4.6$^1$</td>
<td>$^{15}$N$_2$ urea</td>
<td>Ileal</td>
<td>Plasma</td>
<td>21.1</td>
<td>Gibson et al. 1998</td>
</tr>
<tr>
<td>Normal adult</td>
<td>Not given</td>
<td>Lactose-$^{15}$N</td>
<td>Fecal</td>
<td>Urine</td>
<td>0.9</td>
<td>Torrallardona et al. 1994</td>
</tr>
<tr>
<td>Young pigs</td>
<td>2$^1$</td>
<td>$^{15}$NH$_4$Cl</td>
<td>Ileal</td>
<td>Carcass</td>
<td>7.9</td>
<td>Torrallardona et al. 1996</td>
</tr>
<tr>
<td>Minipigs (ileorectal anastomosis)</td>
<td>3.5</td>
<td>$^{15}$NH$_4$Cl</td>
<td>Ileal</td>
<td>Carcass</td>
<td>15.3</td>
<td>Metges et al. 1996</td>
</tr>
<tr>
<td>Young rats</td>
<td>0</td>
<td>$^{15}$N$_2$ urea</td>
<td>Ileal</td>
<td>Plasma albumin</td>
<td>8.2</td>
<td>Torrallardona et al. 1996a</td>
</tr>
</tbody>
</table>

$^1$ g protein·kg$^{-1}·d^{-1}$.  
$^2$ kJ·kg$^{-1}$.  
$^3$ Mean of fasted and fed state; not significantly different between $^{15}$NH$_4$Cl and $^{15}$N$_2$ urea tracers.
of the true precursor enrichment for absorption, it is crucial to address these concerns.

Intestinal absorption of amino acids has been shown to be maximal in the mid-lower jejunum, and human studies with ileal tubes show that at the ileum level, dietary nitrogen is still recovered, suggesting a role of the ileum for complete uptake of dietary amino acids (Gaudichon et al. 1999). This conclusion is supported by the observation that peptide transporters were upregulated in the distal regions of intestine by a high protein diet (Erickson et al. 1995). There is evidence that enterocytes high on the villus are mainly responsible for absorption. In studies of the injection of mRNA from rat small intestine into Xenopus laevis oocytes, expression of three types of lysine transport has been identified (Munck and Munck 1994). The peptide transporter PEPT1 has been localized to the apical microvillus plasma membrane of the absorptive epithelial cells of the rat small intestine and shown to be responsive to nutritional condition (Erickson et al. 1995, Oghara et al. 1999). PEPT1 appears to be exclusively expressed in small intestinal tissues, but no PEPT1 mRNA could be detected in large intestinal tissues of various species (Chen et al. 1999, Doring et al. 1998). However, weak signals of PEPT2 specific fragments have been identified in rabbit colon (Doring et al. 1998), although the importance of this finding for human colon epithelial cells remains to be seen.

M cells, an epithelial cell phenotype that occurs only over organized mucosal lymphoid follicles, deliver samples of foreign material (antigens and microorganisms) via transepithelial transport from the lumen to organized lymphoid tissues within the mucosa of the small and large intestines (Sanonetti and Phalipon 1999). In healthy animals, it is likely that spontaneous bacterial translocation occurs at a low rate but that bacteria are killed by the host immune defense. Hence, it would be theoretically possible that 15N-labeled bacterial material enters the circulating plasma via this pathway as indicated for 13C-labeled E. coli in mice (Gianotti et al. 1995).

Absorption of microbial protein-derived amino acids would require that microbial protein breakdown occurs at the ileum. There is evidence for high proteolytic activity in human ileal effluents due to small intestinal peptidases but also due to bacterial proteases (Macfarlane et al. 1988 and 1989). For some bacteria, peptides derived from microbial protein breakdown and dead and lysed bacteria cells may not be immediately reincorporated into microbial protein but instead released into the surrounding medium (Cotta and Russell 1996). It is interesting to note that peptide-bound amino acids contributed to ~50% to the portal plasma amino acid pool in the rat (Seal and Parker 1991). However, the extent to which these peptides may be derived from microbial protein is not known.

Evidence for absorption of microbial amino acids from small intestine

15N and 14C lysine enrichment of microbial protein after the ingestion of 15NH4Cl and 14C polyglucose changes throughout the gastrointestinal tract of pigs (Torrallardona et al. 1994). No enrichment has been found in the small intestine with the exception of the ileum, whereas a substantial enrichment in the cecum, followed by an increase toward the distal colon comparable to the enrichments in feces, has been observed (Torrallardona et al. 1994). Based on the comparison of enrichments of both isotopes in the digesta and the carcass, it was suggested that microbial lysine has been absorbed in the small intestine. The same authors studied the site of microbial lysine absorption by returning 15N-labeled digesta into the ileum of unlabeled pigs. They found that at least 75% of total microbial lysine absorption occurred in the small intestine (Torrallardona et al. 1996b).

Our investigations in pigs with end-to-end ileorectal anastomosis and patients with ileostomy indicate that microbial amino acids can be synthesized in the small intestine, and their appearance in the free plasma pool indicates their absorption from that site (Metges et al. 1996 and 1999a). However, ileostomy as well as ileorectal anastomosis is prone to secondary colonization and possibly alterations of digesta transit rate in the gut. Hence, although it might be not completely comparable to the microbial situation in an intact gastrointestinal tract, it demonstrates the principal possibility of microbial lysine and threonine absorption from the small intestine.

Table 4

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Free amino acids</th>
<th>Microbial protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Threonine</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.2</td>
<td>3</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.4</td>
<td>19</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.07</td>
<td>3</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.03</td>
<td>0.6</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Calculated from data of Niiyama et al. 1979.

Evidence for absorption of microbial amino acids from large intestine

After cecal administration of 15N-labeled yeast, 15N label appeared in the body protein of infants (Heine et al. 1987). However, the mere appearance does not imply the absorption of intact microbial amino acids. Also, the finding that 30–100% of free lysine, threonine, serine, histidine and arginine from an enzymatic casein hydrolysate solution disappeared from the cecum of pigs (Olszewski and Buraczewski 1978) has to be viewed with caution because the surgical isolation of the cecum and the continued antibiotic lavages generate rather artificial conditions that may not be comparable to normal conditions.

Using 15N-labeled rectum content, the appearance of 15N lysine and other 15N amino acids were detected in the colic branch of the ileocolic vein 3 h after infusion into the cecum (Niiyama et al. 1979). In the same study, it has been shown that the enrichment of amino acids in the digesta differed largely between the free and the protein-bound amino acid pool (Table 4). This observation emphasizes the importance of knowing the true precursor pool for microbial amino acid absorption.

The comparison of the appearances of microbial amino acids in the free plasma pool amino acids after ingestion of the 15NH4Cl tracer when ileal and fecal microbial enrichments are being used (Table 2) suggests a role of the colon in the absorption of nitrogen (Metges et al. 1999a and 1999b). Amino acids undergoing a moderate nitrogen exchange in the
body (valine, histidine) show rather comparable numbers, whereas glutamic acid and alanine, which are known for extensive nitrogen exchange, differ by a factor of 2 between normal subjects and ileostomates, thereby indicating an uptake of $^{15}$N-labeled nitrogen by the large intestine (Table 2). However, this does not shed light onto the identity of the substances absorbed.

On the other hand, several reports indicate that amino acid absorption from the large intestine is negligible in nonruminant animals (Darragh et al. 1994, Hume et al. 1993). However, Fuller and Reeds (1998) recently summarized data on N balance measurements in pigs when protein or amino acids were infused into the large intestine and found that the whole body N balance was always slightly improved. This suggests that there is protein digestion and that there might be absorption of amino acids. However, the identity of the substances absorbed may still be in question because no nutritional benefit was seen when lysine was infused into the colon, although the latter finding does not exclude the possibility of peptide absorption as mentioned earlier.

In conclusion, it appears as if the small intestine is responsible for a large part of microbial lysine uptake, although some absorption from the large intestine cannot be excluded as based on new information on peptide transporters in colon tissue.

Amino acid losses from gastrointestinal tract

Oxidation is the major component of metabolic disposal of IAA and is related to the level of dietary IAA supply, as shown in various stable isotope studies by Young and Borgenha (2000). Other routes of lysine loss have been considered to be minor and thus were not included in the calculation of lysine balance. Lysine oxidation, in contrast to leucine oxidation, is different when given orally and intravenously (El-Khoury et al. 1998), and the intravenous $^{13}$C lysine tracer results in an understimation of lysine oxidation, suggesting that first-pass splanchnic and possibly microbial oxidation contributes to the whole body lysine oxidation rate.

Assuming that there is no quantitative important absorption of amino acids from the large intestine, it is thought that IAA entering the cecum through the ileocecal sphincter are lost to the body. Cummings and Macfarlane (1997) summarize that on mixed European diets, total ileal N is 2–3 g/d, whereas fecal N is 2–4 g/d, suggesting that the colon is in approximate N balance. Daily irreversible lysine losses at the terminal ileum have been estimated from the ileal effluent of subjects on protein-free diets to be 4.6 and 3.9 mg · kg⁻¹ after a 2-d intake of an antibiotic (Fuller et al. 1994). This suggests that under these experimental conditions, the losses are mainly of endogenous origin. When receiving a mixed diet providing ~1 g protein · kg⁻¹ · d⁻¹ in the form of a crystalline amino acid mixture, a daily loss of 8.5 mg · kg⁻¹ has been estimated (Metges et al. 1999a), and a comparative value can be calculated for threonine (Metges et al. 1999b).

Nitrogen sources for microbial amino acid synthesis

Ammonia is the preferred source of nitrogen for the growth of enteric bacteria (Reitzer and Magasanik 1996) but only if there is sufficient ATP to drive microbial protein synthesis. As known from rumen bacteria, acetate and CO₂ and, to a lesser degree, propionate derived from microbial carbohydrate fermentation, form an active precursor pool of carbon for amino acid synthesis (Sauer et al. 1975). Ammonia is assimilated to form glutamate and glutamine (Reitzer and Magasanik 1996), and the glutamate provides nitrogen for the synthesis of most of the other amino acids. Lysine biosynthesis proceeds from aspartate via the intermediate diaminopimelic acid, required by many bacteria for the biosynthesis of cell wall (Morrison and Mackie 1996). Hence, if $^{15}$NH₄Cl is given orally, $^{15}$N-labeled ammonia might be quite rapidly incorporated into microbial amino acids (Metges et al. 1999a, Fig. 4). Furthermore, the $^{15}$N ammonia can reach the liver directly via the portal vein or transferred through the intestinal wall where it is introduced into dispensable (mainly arginine, glutamine) and also some IAA via transamination as well as being transferred to be incorporated into urea. In this context, it is interesting to note that in our study in healthy adult subjects, only 38.7% of the $^{15}$N ingested as $^{15}$NH₄Cl was excreted as urinary urea, whereas the isonitrogenous dose of $^{15}$N urea resulted in a significantly higher fractional excretion of 56.7% (Metges et al. 1999b).

The $^{15}$N from non-specific nitrogen sources is also returned to the intestinal tract as $^{15}$N-labeled amino acids and as $^{15}$N urea in endogenous secretions (pancreatic, biliary and mucosal) (Fuller and Reeds 1998). Intravenous infusion of $^{15}$N-labeled amino acids is followed by labeled plasma amino acids, appearing in the gastrojejunal fluids within ~3 h after onset of the infusion (Gaudichon et al. 1994). Leterme et al. (1996) showed in pigs that the tracer appears in pancreatic secretions within 50 min of the consumption of a $^{15}$N-labeled diet. This finding is confirmed by our observations in the pig showing substantial $^{15}$N enrichment of amino acids (i.e., lysine, alanine, glycine, leucine, isoleucine and glutamic acid) in duodenal and jejunal proteins after a 10-d administration of $^{15}$NH₄Cl (Metges et al. 1996, C. C. Metges, unpublished data). The quantity of endogenous protein that is recycled into the intestine makes it a potentially significant source of nitrogen for microbial growth. Quantitative information comes from experiments in growing pigs in which digesta from the upper intestinal tract was transferred between one pig fed $^{15}$N and another that was not (Krawielitzki et al. 1990). This study suggests that 90% of all endogenous nitrogen secreted into the gut is reabsorbed, although the experiments do not throw any light on the involvement of the enteric flora in this process. However, because mucus glycoproteins and some other digestive secretions are resistant to mammalian digestive enzymes, microbial proteolytic activity is involved (Macfarlane et al. 1988, Quigley and Kelly 1995).

Approximately 60–70% of newly synthesized urea is excreted in the urine, whereas the remainder is degraded by microbial urease. It has been claimed that urea nitrogen can be salvaged in the colon and that this nitrogen can be incorporated by the intestinal microflora into amino acids that are subsequently absorbed by the host (Jackson 1993, 1995). Bacterial growth on sources of nitrogen other than ammonia is also possible but slower than with ammonia because the rate of ammonia generation from these sources (i.e., amino acids or urea) appears to be a growth-limiting factor (Reitzer and Magasanik 1996). In rats, urease activity in small intestinal contents (units/g collected content) was 13% compared with that found in the large intestine (Kim et al. 1998). As shown for the human colon and the bovine rumen, ureolytic bacteria seem to be mainly located close to the intestinal walls (Hume 1996), which might explain why there was only low enrichment of cecal luminal ammonia when $^{15}$N urea was infused intravenously into human subjects (Wrong et al. 1985). When urea is intravenously infused into pigs, urea concentrations in the jejunal and in the colonic perfusate increase significantly. In contrast, ammonia concentration measured in the same animals was not significantly changed (Malmlof and Simoes
Acids: the 15N enrichments of microbial amino acids were only the differences were far smaller than those for plasma amino acids we measured, lysine, threonine and histidine, were more not influence the composition or density of the nonpathogenic species. However, this does not mean that these factors could microflora have been mainly focused on pathogenic microbial nitrogen at the human terminal ileum (2–3 g protein). Chacko and Cummings (1988) report that total nitrogen secretions, bacterial protein and undigested food there also is an ample supply of amino acids from small intestine, although nitrogen source for microbial protein synthesis is of greater significance in the colon than in the small intestine, although aspartate, arginine and NH₄Cl was observed (Wu 1995), which points to the possibility of a tightly connected nitrogen recycling between juxtamucosal ureolytic bacteria and the enterocyte.

Furthermore, a comparison of the intake of isonitrogenous amounts of ¹⁵N₂ urea and ¹⁵NHCl in healthy human subjects (Metges et al. 1999b) showed that the degree of ¹⁵N labeling in the microbial amino acids was, as for plasma free amino acids, higher with ¹⁵NH₄Cl than with ¹⁵N₂urea. However, the differences were far smaller than those for plasma amino acids: the ¹⁵N enrichments of microbial amino acids were only approximately twice as high with ammonium chloride as with urea, whereas free dispensable amino acids and leucine and valine in plasma were between 10 and 20 times higher after ¹⁵NH₄Cl than after ¹⁵N₂urea. In contrast, the other IAA that we measured, lysine, threonine and histidine, were more equally labeled with ¹⁵N from the two sources, although the enrichment was still higher with ¹⁵NH₄Cl (Metges et al. 1999a and 1999b). This indicates that an oral dose of urea is a relatively more effective source of N for microbial amino acid synthesis than it is as a source of N for the synthesis of tissue endogenous amino acids.

Thus, taken together, it is reasonable to assume that microbial amino acid synthesis in the human gastrointestinal tract uses a mixture of various nitrogen sources, i.e., ammonia derived from amino acids and urea. Because urea hydrolysis is dependent on the availability of microbial urease activity, which is apparently lower in the small intestine than in the colon, microbes in the small intestine may rely more on endogenous amino acids and ammonia from gastrointestinal secretions.

At least in the pig, the upper digestive tract (stomach and small intestine) represents the main site of urea secretion (Malmlof and Simoes Nunes 1992, Mosenthin et al. 1992 ). The possibility of urea diffusion through the colon wall might be difficult to detect, assuming that the major part of urea hydrolysis and utilization for microbial amino acid synthesis takes place juxtamucosal. In any case, it appears that urea as a nitrogen source for microbial protein synthesis is of greater significance in the colon than in the small intestine, although there also is an ample supply of amino acids from small intestinal secretions, bacterial protein and undisgested food protein. Chacko and Cummings (1988) report that total nitrogen at the human terminal ileum (2–3 g · d⁻¹) consists of 10–15% urea/ammonia/nitrate and free amino acids, 48–51% protein and 34–42% peptides.

Dietary effects on intestinal microbial IAA synthesis

Reports on the effect of dietary factors on the intestinal microflora have been mainly focused on pathogenic microbial species. However, this does not mean that these factors could not influence the composition or density of the nonpathogenic indigenous gut microflora, and thereby possibly the microbial protein synthesis might be affected.

Deprivation of nutrients such as glucose can induce the formation of adhesive organelles to enable bacteria to gain access to nutrient sources within or adjacent to host cells (reviewed by Alverdy and Stern 1998). It was shown that gram-negative bacteria establish glycocalyx-coated microcolonies on epithelial cells during periods of luminal nutrient deprivation (reviewed by Alverdy and Stern 1998), and it could be speculated that feeding purified or chemically defined diets such as used in a number of experiments investigating microbial lysine utilization with low contents of fermentable carbohydrates (e.g., Giordano et al. 1968, Metges et al., 1999a) might have changed the microbial environment. Chemically defined liquid diets fed to rats for 1 week resulted in overgrowth of coliform strains and altered bacterial translocation from the gut (Alverdy et al. 1990). It was also observed that in humans, overall microbial cell counts decreased by ~10–20% when purified diets were ingested (Blaut, M., personal communication). On the other hand, overall dietary restriction (60% of ad libitum food intake) had little effect on the faecal microflora of female Fischer 344 rats (Henderson et al. 1998). However, short-term starvation induced a 7500-fold increase in E. coli bacteria adherent to cecal epithelium in mice (Hendrickson et al. 1999).

Dietary fat content could also have an impact on microflora because reports indicate bactericidal effects of various fatty acids and monoglycerides on gram-positive bacteria (Petschow et al. 1998, Sprong et al. 1999). Feeding rats diets containing milk, yogurt, lactose or cellulose considerably decreased urea activity and ammonia production (Kim et al. 1998), which was thought to be due to the growth of nonurea, nonammonia producers, such as lactobacilli for the milk product–based diets.

Numerous studies have shown that the ingestion of certain nonstarch oligosaccharides can affect the composition of the microflora and subsequently of their fermentation products (e.g., Hylle et al. 1998, Macfarlane and Cummings 1999). However, this goes beyond the scope of the present review, and the reader is referred to the relevant literature.

Using the ¹⁵N labeling paradigm in a recent study in minipigs, our preliminary results indicate that microbial lysine isolated from ileal chyme is apparently more ¹⁵N labeled when pigs were adapted to a low lysine but otherwise adequate diet (Backes et al., unpublished data). It has been shown in growing pigs that there is an adaptation to lysine amino acid deficiency in that lysine concentration in whole body protein decreases whereas other amino acids are more concentrated (Batterham et al. 1990). Amino acid compositions of endogenous nitrogen secretions are dependent on the protein status of the animal (de Lange et al. 1989). Hence, it is possible that lysine content or other nitrogenous compounds of endogenous secretions in lysine-deficient animals are lower than in animals adequately supplied with lysine, which could lead to changes in the intestinal microenvironment.

Feeding diets containing 20% protein compared with 5% to septic and control guinea pigs resulted in increased bacterial translocation measured by the instillation of ¹⁴C-labeled E. coli, with nonsignificantly higher counts in the liver of control animals (Nelson et al. 1996). In addition, mice fed glutamine-enriched diets had a lower degree of translocation to the tissues (liver, spleen and lymph nodes) (Gianotti et al. 1995).

These results indicate that the intake of certain dietary oligosaccharides, lipids, milk products as well as protein intake and the level of consumption of certain amino acids can affect the composition or metabolic activity of the intestinal microflora and thus its fermentation products potentially available to the host.
Relevance for determining adult IAA requirement

Using the $^{15}$N labeling paradigm, it could be shown that microbial lysine is used for lysine homeostasis in normal human subjects. However, to define the net contribution of microbial IAA to meet the human IAA requirement, the $^{15}$N labeling paradigm is not the ultimately successful experimental strategy because interpretation of results is complicated by the nitrogen recycling into and from the gut. To quantify the net contribution of microbial amino acids to meet the metabolic demand for IAA would require that the material used to produce microbial IAA would be otherwise lost or of no further value for the body. Hence, to the extent that the growth of the microbes that give rise to the labeled lysine is supported by the degradation of endogenous protein, then the appearance of microbially derived lysine in plasma can be seen as part of the normal mechanism by which endogenous nitrogen and amino acids are recycled rather than microbial amino acids serving as a net source of amino acids, which are additional to those supplied in the diet (Metges et al. 1999a). Furthermore, if $^{13}$N-labeled substrates entering the gut lumen (whether plasma-derived amino acids, endogenous proteins or urea) were used preferentially by microbes in juxtaposition to the intestinal wall with turnover and release of their constituent proteins and amino acids in that spatial domain, then the $^{15}$N enrichment of lysine being absorbed from the gastrointestinal tract would not be accurately reflected by either the ileal or fecal microbial protein-bound lysine.

In a pig, experiment $^{13}$C polyglucose was used as a tracer to label microbial lysine (Torrallardona et al. 1994), and it might appear that this could have solved the problems related to nitrogen cycling. The authors estimated the microbial lysine contribution to body lysine based on cecal enrichment to be 0.2% compared with $\sim 1\%$ estimated using the $^{15}$N labeling paradigm in the same animal. However, the discussed difficulties in interpreting the results using a carbon-labeled tracer do persist, although to a lesser degree, for very similar reasons. First, the difficulty in selecting the true precursor pool (i.e., small or large intestine, luminal or juxtapostal, free or protein/peptide-bound amino acids) would remain. Second, it cannot be excluded that carbon derived from carbohydrates that are not readily accessible by the enzymes of the host might return into the gastrointestinal tract as IAA via endogenous secretions and sloughed-off cells (Simon et al. 1986) and thus be reincorporated into microbial protein. Simon et al. (1986) estimated for a 34-kg pig that in 12 h, 0.79 g leucine was secreted into the upper gastrointestinal tract derived from the plasma pool. The possibility of using $^{13}$C-labeled polyglucose instead of $^{14}$C in humans would require a comparably large amount (50–100 g) of highly uniformly labeled non-starch polysaccharide (with the disadvantage of severely affecting the normal gastrointestinal transit rate) to be able to detect $^{13}$C-labeled lysine in the plasma pool.

When introducing a potential microbial IAA contribution to the input side of the tracer balance equation, a potentially irreversible loss of IAA from the human gastrointestinal tract must also be considered. A microbial contribution to the input side only would overestimate the dietary supply if a potential gastrointestinal loss is not accounted for. It is therefore possible that the microbial contribution and the gastrointestinal losses cancel themselves out.

In conclusion, we and others have shown that there is a significant presence of microbially derived lysine in the body protein and plasma pool of humans and nonruminant animals. However, the current use of the $^{15}$N labeling paradigm does not allow an estimation of the net microbial contribution because of the various uncertainties, as discussed.

LITERATURE CITED


