Nitrogen Trafficking and Recycling Through the Human Bowel

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Normal adults maintain nitrogen balance over long periods, and during growth in childhood there is modest positive nitrogen balance. Nitrogen is consumed mainly as protein in the diet, and is made available to metabolism as amino acids, following digestion and absorption. Normally amino acids contribute about 20% to the energy requirements of the body, as well as being used for synthetic processes [1]. A consequence of the oxidation of amino acids is that the amino group is lost to the system, mainly through the formation and excretion of urea. Of the urea produced, the proportion which is excreted in urine varies depending on the overall metabolic state, the energy and protein consumption of the individual, and the metabolic activity of the colonic microflora. From 25 to 90% of the urea nitrogen produced may be retained in the system following hydrolysis by the colonic microflora, thereby playing a fundamental role in achieving nitrogen balance and making a significant contribution to the nitrogen economy of the body [2–4]. Thus the gastrointestinal tract plays a central role in protein, amino acid and nitrogen metabolism, firstly by making dietary protein available as amino acids to metabolism, and also by participating in the processes whereby nitrogen balance is achieved under a range of dietary and physiological circumstances. In this chapter I consider the quantitative and qualitative aspects of the movement of protein amino acids and nitrogen through the bowel, and conclude that the small intestine and large intestine operate as functionally distinct entities.

The detailed nature of the movement of nitrogen in and around the bowel is complex, and beyond our present ability to describe in a satisfactory way. In order to be in a position to start investigating the nature of the exchanges which take place we have to start with certain assumptions. It is axiomatic that in considering the relative importance and role of nitrogen and nitrogen exchange,
Nitrogen Trafficking and Recycling Through the Human Bowel

the objective is to provide a suitable mix of a range of amino acids that can adequately meet the needs of the body as the building blocks for protein synthesis and as the fundamental precursors for a range of critical metabolic pathways; elementally, this is what differentiates protein from the other macronutrients. Other contributors to this volume give detailed expositions of the ways in which function might be explored in human and animal models, and the single theme which unifies all this work is the complexity and relative inaccessibility of the small and large intestine for detailed investigation. The mucosa itself is highly active metabolically, and there is considerable interaction with intraluminal events, which are themselves extensive and complex. This complexity is increased further in the colon because of the presence of a florid microflora, which is estimated to have more cells than the host by an order of magnitude, and the metabolic capacity of the liver [5, 6]. There is clearly very extensive interaction between host and bacterial metabolism, and yet bacterial metabolism appears beyond any recognized mechanisms of metabolic control. To a large extent our understanding of detailed events is constrained by the complexity of the system, and its relative inaccessibility for investigation. What is clear is that the net changes, identified as the balance of inflow and outflow, is too crude a measure of the extent of change and an insufficient statement of the nutritional interchange which takes place. The magnitude of the internal flux is very much greater, and therefore we have to be prepared to use a stochastic approach and deductive reasoning about the nature and magnitude of the changes that are taking place within the ‘black box’ to advance our understanding. More than any other place in metabolism we have to take care to differentiate between the movement of nitrogen and that of amino acids or protein.

Quantitative Flow of Nitrogen

Functionally the gastrointestinal tract can be considered to consist of two separate organs, the small intestine and the large intestine (Fig. 1). They appear to operate in series, but in terms of the flow of nitrogen they can be considered to be functionally separate to a large extent. Much of metabolic integration in nutrition is determined by how the demands imposed by the needs of individual tissues and organs for energy and nutrients can be adequately met. For the entire bowel, there are important barrier, inflammatory, and immune functions that impose metabolic demands. However, for the small intestine the most obvious quantitative function is the need to process the food ingested and following the digestion and absorption of the products of digestion, to make energy and nutrients available to the system. Function appears to be driven by the presentation of luminal material from the food consumed, and therefore the functional demand is determined by the quality and quantity of the material consumed. As discussed elsewhere in this volume, these processes take place at considerable metabolic cost, and the flux of nitrogen associated with the production of
Nitrogen Trafficking and Recycling Through the Human Bowel

**Fig. 1.** For both the small intestine and the large intestine, the luminal flow of protein, amino acids, and nitrogen is far greater than would be appreciated simply from the net change across either lumen. Although the small and large intestines are in direct continuity, there is only limited mixing of the nitrogen compounds which flow through either. The activity in the small intestine is determined by the magnitude of the supply from the diet, whereas for the large intestine the flow of nitrogenous materials is determined by the metabolic activity of the resident microflora and the metabolic demands of the host. The flow of urea nitrogen through the bowel is a small part of the total flux.

enzymes, secretions which include mucins, and cellular turnover is at least as great as that derived from ingested protein (Fig. 1). Thus in a normal adult the total flow of nitrogen through the small intestine is estimated to be in the region of 26–30 g N/day. Notwithstanding the magnitude of this flow, absorption is very efficient with 90–95% recovery by the time the contents have reached the ileocecal valve [7–11].

On the basis of intubation studies, the flow of nitrogen through the ileocecal valve is of the order of 2–3 g N/day, and losses of nitrogen in stool are between 1 and 2 g N/day [2]. This has led to the presumption that the flow of nitrogen through the colon is not very great. However, if the dilution of isotope which passes into the colon, or which is placed in the colon, is followed, the estimated flow of nitrogen through the colon would be around 14–16 g N/day [12, 13]. These represent very considerable flows of nitrogen, especially when compared with the rates of whole body protein turnover.

**Qualitative Changes**

**Small Bowel**

As pointed out by Reeds in this volume, tracer studies show that there are important differences in the metabolic handling of nutrients presented through either luminal or systemic routes [14, 15]. Much of the information on the
Nitrogen Trafficking and Recycling Through the Human Bowel

qualitative aspects of the movement of nitrogen around the small bowel has been derived from measurements of mass balance, or by following the fate of the carbon skeleton of amino acids. There are few data examining the partitioning of nitrogen directly, and the most extensive of which I am aware are those reported within a thesis by Jahoor [16]. Following the intravenous infusion of individual $^{15}$N-labeled amino acids, enrichment was determined in the amino acids within the free pool of the small intestine of fasted rats (Fig. 2). Labeled nitrogen was infused as ammonia, glutamine-amide-N, glutamic acid, alanine, aspartic acid, lysine, and glycine, and enrichment measured in urea, ammonia, glutamine-amide-N, glutamic acid, alanine, and aspartic acid. The enrichment in urea was very similar following the infusion of most of the amino acids, although after an equimolar amount of glutamine the level of enrichment was twice as great as the average, and for both lysine and glycine less nitrogen was recovered in urea than might have been expected.

The implications of this selective movement of amino acid nitrogen to urea have been considered in the context of measuring whole body protein turnover and the relative suitability of one or other $^{15}$N-labeled amino acid, but never really considered seriously in relation to the economy of whole body nitrogen [17–19]. For ammonia and glutamine, there appeared to be ready exchange of nitrogen, but for each the distribution of nitrogen to other amino acids was strictly limited. Similarly, there was exchange of nitrogen among the transaminating amino acids – alanine, glutamic acid, and aspartic acid – with only limited exchange beyond the three amino acids. Remarkably it was difficult to identify much movement of the nitrogen from aspartic acid to other amino acids, and even the recovery of label in aspartic acid was very low. For glycine and lysine, which are known not to participate in nitrogen exchange reactions to any great extent [18, 20], there was less label identified in any one amino acid than in urea itself. To an extent these results are unremarkable, and fit with observations made when the distribution of nitrogen was determined from the enrichment of individual amino acids in proteins, following the administration of different labeled amino acids [20]. However, what is important is that this represents the first time that enrichment has been measured in the free amino acid pool, and the first time that this has been reported for the gastrointestinal tract specifically. It emphasizes that amino nitrogen does not move freely among amino acids, and is strictly channeled. The amino acids might be considered to fall into distinct functional groups in respect to this partitioning, for which the simplest division would be into deaminating and transaminating amino acids [2, 21].

Despite the limitations in the approach used, the data show that the flow of nitrogen among amino acids and to the end products of metabolism is channeled and very different from what might be assumed from classical biochemical studies.
Fig. 2. Rats in the postabsorptive state received intravenous infusions of tracer amounts of $[^{15}N]$-labeled compounds for 6 h, and the enrichment in the free amino acid pool of the cleansed gut was determined. (a) The enrichment in the urea pool of the gut, following infusion of either ammonium chloride, glutamine, glutamic acid, alanine, aspartate, lysine, or glycine on separate occasions is shown. (b–c) The enrichment in ammonia, glutamine, glutamic acid, alanine, and aspartic acid (normalized to an enrichment in urea of 100) following the infusion of labeled ammonium chloride and glutamine (b), glutamic acid, alanine, or aspartic acid (c), or lysine and glycine (d) in different studies. Data taken from Jahoor [16].
Nitrogen Trafficking and Recycling Through the Human Bowel

Large Intestine

Compared with the small intestine, the large intestine is very different functionally, and the metabolic activity of the cecum and colon appear to be dominated by the metabolic requirements of the bacteria. However, this appearance overshadows the very important role played by the colon in allowing metabolic adaptation in the host, and there are three issues of importance in the context of present considerations. First, it seems that colonic function is an integral part of the adaptive response of the host, both to protein and to energy [22–25]. Second, for the colonic microflora, the availability of nondigestible carbohydrate appears critical to the way in which nitrogen is handled, the flow of nitrogen through the colon being five to ten times greater than the appearance of nitrogen in stool, but the data are difficult to come by [5, 13]. Third, the host’s ability to interact with the bacteria may be determined by their own metabolic demands, any increase in the efficiency with which dietary nitrogen is used reflecting the ease with which urea nitrogen can be salvaged following hydrolysis by the colonic microflora [2, 22]. It appears that the net result of the interaction between the host and its microflora is that the products of bacterial metabolism contribute to the dietary intake by helping to match the metabolic demands of the host, both quantitatively and qualitatively. There are few data enabling us to derive quantitative estimates of the movement of nitrogen through the colon, but it is possible to infer a considerable amount by following the fate of nitrogen which passes to the colon as a part of the process through which urea nitrogen is salvaged.

Urea Kinetics

The ability to achieve nitrogen balance across a wide range of protein intakes is to a large extent determined by changes in the rate at which urea is excreted in urine to match the nitrogen consumed in the diet [1, 4, 26]. On the basis of biochemical experiments and studies in which the dietary manipulations have tended to be extreme, it has been concluded that the changing rates of urea excretion follow changes in the rate at which urea is produced. More recently it has become clear that the rate of urea production is remarkably constant over the daily cycle, from day to day, and across a very wide range of protein intakes. Variability in urea excretion is the consequence of differences in the partitioning of urea, between the urine and the colon. As protein consumption falls from the habitual levels of 70–90 g/day to lower levels, a decreasing proportion of production is excreted in urine, with an increasing proportion passing to the colon [1]. It appears likely that this is in part a controlled process, through conjugate regulation of the activity of the UT2 transporter in the collecting duct of the kidney and the colon [1]. Salvage of urea nitrogen increases as protein intake is reduced, down to the level of the physiological minimum intake of protein, about 35 g/day in normal adult males. When the consumption of protein
falls below the physiological minimum requirement, nitrogen balance cannot be sustained, there is a reduction in the rate of urea production and an increase in the rate of urea excretion in the urine, and urea salvage falls [26, 27]. Maintaining the rate at which urea is produced appears to be critical for maintaining the activity of the urea salvage mechanism; thus if urea is added to the intake of a person consuming a diet that contains protein at a level below the physiological minimum requirement, the salvage of urea nitrogen is increased and nitrogen balance improved [27]. Thus both in terms of studies using urea mass balance and those in which urea kinetics have been determined, the effective salvage of adequate amounts of urea nitrogen is critical to achieving nitrogen balance on diets low in protein [2, 28, 29].

There is evidence that at very high levels of protein consumption, beyond the range of habitual intakes, there is a significant increase in the rate of urea production and excretion, but the level of consumption at which this increase occurs has not been clearly defined [30, 31].

For many years it has been known that for individuals consuming a diet low in protein and in negative nitrogen balance, the addition of nonessential nitrogen to the diet can restore nitrogen equilibrium [32]. Thus during infancy the addition of glycine or urea to an inadequate diet enhances growth and nitrogen balance [33]. For adults, a range of sources of nonessential nitrogen might be used, with differing efficiency [2]. Urea is not a particularly good source of nonessential nitrogen, but fulfills the function under suitable circumstances. As urea nitrogen cannot be used directly, effective use must follow hydrolysis by the colonic microflora. The most direct evidence that the colonic salvaging of urea plays a critical role in nitrogen homeostasis comes from those studies in which people who are in negative nitrogen balance on a diet low in protein can be brought into balance by the adding urea nitrogen to the diet, and stimulating urea nitrogen salvage in the bowel [27–29, 34]. On the basis of the kinetic data, the movement of urea into the bowel ranges from 3 g N/day on higher protein intakes to 6 or 7 g N/day when dietary protein is marginal, equivalent to about 40 g protein/day.

An unreliable picture is obtained of the extent of colonic nitrogen exchange from those studies which base their interpretation on the loss of nitrogen in the stool. Following oral or intravenous administration of labeled urea [12, 13], or the direct placement of labeled material in the colon, the level of enrichment in stool is very low [35, 36]. There is considerable dilution of label derived from the urea which passes from the blood into the colon [12]. Therefore the nitrogen derived from urea in the colon is mixing with a much larger pool of nitrogen which is unlabeled, and the evidence would suggest that urea contributes around 15–25% of the nitrogen in this pool, indicating a flow of nitrogen through the colon equivalent to 16–18 g/day. This is a very considerable metabolic activity which has almost completely escaped the attention of those with an interest in protein, amino acid, and nitrogen metabolism.
Quantitative Considerations

It is difficult to gain access to the colon for investigation and most studies have taken advantage of people who are being investigated for other purposes, where the function of the bowel cannot be presumed to be normal, or where cleansing of the bowel has been a prerequisite. The extent to which colonic function changes in response to different conditions has often, therefore, had to be inferred from indirect measurements. Most direct access can be achieved in individuals with a colostomy. Heine et al. [37] showed that, when yeast protein was placed in the colon through a colostomy in infants, <5% of the label was subsequently recovered in stool, and a similar small proportion was recovered from the urine. Therefore it could be concluded that the label was rapidly absorbed and retained in the body. We cannot know the form of the label at the point of absorption and it remains possible that it was absorbed as protein, peptides, amino acids, or ammonia, but whatever the form, it seemed to disappear from the lumen relatively quickly. We have used a similar approach to trace the fate of $^{15}$N$_{$_{15}$}$urea placed directly in the colon, either through a colostomy or directly into the right or left colon at colonoscopy [35, 36]. We hypothesized [2] that the material had one of five possible fates:

- It could be lost in the stool;
- It could be absorbed intact and lost in the urine as $^{15}$N$_{$_{15}$}$urea;
- It could be hydrolyzed by the microflora and absorbed as $^{15}$N ammonia and subsequently reformed to $^{15}$N$_{$_{14}$}$urea and lost in the urine;
- It could be hydrolyzed by the microflora and absorbed as $^{15}$N ammonia or fixed as amino acids in the liver;
- It could be hydrolyzed by the microflora and following hydrolysis the $^{15}$N could be used by the bacteria as a source of nitrogen for their own metabolism with the production of $^{15}$N labeled products, which might be available to the host.

Figure 3 shows that only a small proportion of the nitrogen was recovered in stool, or in urine as either $^{15}$N$_{$_{15}$}$urea or $^{15}$N$_{$_{14}$}$urea. The majority was retained within the body and therefore presumably fixed either through bacterial metabolism or that of the host. A significantly greater proportion of the dose of label was recovered as $^{15}$N$_{$_{15}$}$urea in urine when the dose was placed in the defunctioned colon, suggesting that the colonic mucosa is readily permeable when urea is presented for absorption, though normally urea is rapidly hydrolyzed by an active microflora. The difference in recovery of label from the defunctioned left colon and the right functioning colon might arise if the left and right colon were functionally distinct, but the finding of similar excretion of $^{15}$N$_{$_{15}$}$urea for label placed in the left or right functioning colon suggests that any differences observed between the two sides was more related to functional state than to intrinsic differences between the left and right sides. For the defunctioned left colon, the recovery of $^{15}$N$_{$_{14}$}$urea in urine and the high
Nitrogen Trafficking and Recycling Through the Human Bowel

**Fig. 3.** The effect of adding fiber to the diet is to increase the wet weight of stool and the nitrogen content of stool, whether the fiber is derived from banana [26] or from wheat or oats [41].

level of retention of label indicate bacterial action within the defunctioned colon, possibly using mucin and sloughed material as a source of energy. It is known that ammonia passing to the liver through the portal vein is preferentially used for urea formation. Therefore, the finding that relatively little of the label appeared as $^{15}\text{N}^{14}\text{N}$urea in urine argues against the label being absorbed as ammonia in any substantial amounts. The explanation which would most readily explain the retention of label from $^{15}\text{N}^{15}\text{N}$urea would be that it had been incorporated
Nitrogen Trafficking and Recycling Through the Human Bowel

into bacterial metabolites, mainly amino acids, which were then available to the host. When a comparison was drawn between the fate of $[^{15}\text{N}]$urea placed in either a functioning or defunctioned colon, the material in the defunctioned colon was less likely to be retained and more likely to be excreted, arguing in favor of bacterial fixation playing an important role.

The other noninvasive approach that has been used recently to assess the handling of urea in the colon in normal adults has been to give lactose-ureide orally. Lactose-ureide is resistant to the normal digestive process and therefore passes to the colon as the complete molecule, where it is subject to bacterial fermentation to release lactose and urea, the urea subsequently being hydrolyzed [38]. In this way a defined amount of urea might be delivered to the colon and if the urea is suitably labeled, the fate of the nitrogen or carbon moiety can be followed [39]. This method has been used to assess gastrointestinal transit time, but can also be used to determine the metabolic fate of urea nitrogen. We have determined the relative fate of an oral dose of lactose-$[^{15}\text{N}]$ureide in normal adults consuming a low-protein diet [40]. Twenty-two percent of the label in the oral dose was recovered in stool, of which 3% was recovered as bacterial protein. About 5% of the oral label was recovered in urine as the intact urea molecule, and 24% as urea which had been formed from the nitrogen derived from hydrolyzed urea. Thus about 73% of the available labeled nitrogen was absorbed, and of that absorbed two thirds was retained within the system. These results can be compared with those obtained by Wutzke et al. [39] in normal adults consuming their habitual protein diet (presumably around 70–90 g protein/day). The circumstances of the two studies are different, but when the consumption of protein was higher the hydrolysis and retention of urea nitrogen appeared lower [39], with higher retention of urea nitrogen when protein consumption was lower [40].

The results from the studies using oral lactose-ureide fit with the data derived from the measurement of urea kinetics, and where the fate of labeled urea placed directly in the colon has been determined. The extent of urea hydrolysis and the salvage of urea nitrogen are influenced by the metabolic state of the host, but of the nitrogen that is derived from urea hydrolysis, between 50 and 70% is likely to be retained within the host, presumably in a metabolically useful form. Indeed this may be an underestimate, as any urea nitrogen that is incorporated into proteins which have a short half life is unlikely to be included.

Apart from the host, the other metabolic demand to be satisfied is that of the bacteria. There is much evidence that an increased consumption of non-starch polysaccharides by the host is associated with an increase in stool mass and stool nitrogen, which in part involves an increase in the biomass of the stool. This implies that the availability of energy is limiting for the growth of the colonic microflora, but when suitable sources of energy are readily available nitrogen will be required to increase an increase in biomass and metabolic activity. Figure 4 shows the effect on stool mass and stool nitrogen when the consumption of dietary fiber intake is increased – in bananas, wheat, or oats – and the associated influence
Nitrogen Trafficking and Recycling Through the Human Bowel

Fig. 4. The fate of \(^{15}\)N was determined after instilling a tracer dose of \(^{15}\)N\(^{15}\)N urea through a colostomy into the right functioning colon, the left functioning colon, or the left defunctioned colon. Although \(^{15}\)N was recovered in stool and urine, as both \(^{15}\)N\(^{15}\)N urea and \(^{15}\)N\(^{14}\)N urea, a large proportion of the dose was retained within the body [36].

upon stool nitrogen, part of which can be directly attributed to increased bacterial mass and bacterial protein or nitrogen [26, 41]. The source of nitrogen to satisfy the increased colonic biomass is not fully understood, but there is evidence that increased utilization of host urea might be important [42]. These data reinforce the evidence obtained in the studies when oral lactose-ureide was given (described above), and 3% of label was recovered in the bacterial fraction of the stool.

Qualitative Considerations

The amount of nitrogen that is made available from the hydrolysis of urea in the colon may be substantial. Even if one takes the minimum on an adequate protein intake, the average rate of hydrolysis is 3–4 g N/day, which may double to 7 g N/day on low intakes. Based on the above discussion, it is likely that around 60–70% of this nitrogen is retained by the host (equivalent to 15–30 g protein/day), and the critical question is, in what form? There are earlier studies suggesting that some of this nitrogen might be used for the formation of essential as well as nonessential amino acids [2, 43, 44]. These proposals have generally been greeted with a considerable degree of skepticism. The major constraint in accepting the veracity of the evidence appears to be the extent to which bacterially derived amino acids might be absorbed across the colon, rather than that bacteria form amino acids from the available nitrogen for their own use. In
this respect, studies in experimental animals – such as the rat which practices refection, or the pig, in which the small intestine is relatively heavily contaminated – do not provide a great deal of help [45, 46]. There are four studies in humans which have attempted to address this problem more directly. Each of these is based on the knowledge that lysine and threonine are two amino acids which do not engage in nitrogen exchange reactions and for which there is an absolute dietary requirement [47–50]. If [15N]-label from any source were to be incorporated into lysine isolated from the host, this would provide very strong evidence for bacterial formation. Based on a study in which urea kinetics were measured in children recovering from severe malnutrition, we have shown that label can be recovered in lysine isolated from urine following the oral administration of tracer doses of [15N15N]urea, indicating that nitrogen derived from the hydrolysis of urea has been incorporated into lysine by bacterial metabolism, and subsequently made available to the host [48]. Furthermore, there was a direct relation between the extent of urea hydrolysis and the level of incorporation of label into lysine. Metges et al. [49] have carried out similar studies in adults and shown that label from ammonia or urea can be recovered in lysine isolated from blood. These studies argue strongly that labeled nitrogen can be incorporated into lysine. The weight of circumstantial evidence suggests that this occurs through the metabolic activity of the colonic microflora. It does not prove conclusively that the activity takes place in the colon, nor does it explain how any amino acids formed by the bacteria cross the wall of the large intestine to reach the systemic circulation of the host. The studies in which normal adults were given oral doses of lactose-ureide have already been outlined above. In the study by Gibson et al. [47], bacterial proteins were isolated from the fecal biomass; the enrichment of [15N]lysine from hydrolyzed bacterial protein was then compared with the enrichment of lysine isolated from urine to obtain a quantitative estimate of the degree to which nitrogen derived from urea presented directly to the large bowel was incorporation of into bacterial protein.

As there are good measures of the plasma flux of lysine, it is possible to determine the relative contribution of bacterially derived lysine to the plasma flux of the host. In men and women, plasma lysine flux is on average about 100 µmol/kg-h, and of this 8% is derived from the lysine formed in the bowel using nitrogen derived from urea. On a daily basis this contribution amounts to around 25–30 mg lysine/kg [47]. The FAO/WHO figure for the dietary requirement for lysine, based upon extensive nitrogen balance studies, is 12 mg/kg/day [51]. In contrast, however, Young’s group have determined the physiological or metabolic requirement for lysine based on the rate at which it is used in the body, and have derived a value of 30 mg/kg-day [52, 53]. There appears to be an error in the estimate of dietary requirement, but the presumption has been that there is no mechanism whereby lysine might be formed in the body. The work outlined above suggests that bacteria may make an important contribution to satisfying the apparent shortfall. If this were true, it would mean that if colonic lysine formation were impaired, then the dietary requirement for lysine
Nitrogen Trafficking and Recycling Through the Human Bowel

would be increased. Conversely, if colonic formation were increased – through, for example, the consumption of greater amounts of nondigestible carbohydrate – the dietary requirement for lysine would decrease. These observations may help to explain the apparently wide variation in the estimates of dietary requirements for essential amino acids which have been obtained in different studies where subjects have been exposed to different conditions. The data imply that the dietary requirement might vary depending on the activity of the gut microflora. If the formation of amino acids by the bacteria in the bowel provides an important source for the host then exploring the wider implications offers interesting possibilities for therapeutic intervention.

The main objection to the idea that bacterial amino acids might be available to the host is the difficulty in identifying suitable transporters for the absorption of amino acids from the large bowel outside the neonatal period. It is necessary to review this position, and the recent demonstration of the presence in the colonocyte of the message for an oligopeptide transporter is potentially important [54]. There is now a need to determine directly whether dipeptides are absorbed from the colon in functionally significant amounts, and the conditions under which this might take place.

**Overall Balance of Amino Acids Required for Normal Metabolism**

It has been shown that once the requirements for essential amino acids have been met in the diet, the effectiveness with which they are utilized is determined by the amount of nonessential amino acids, or nonessential nitrogen [2, 33]. Different sources of nonessential nitrogen can fulfill this role with varying degrees of efficiency. The general rule is that the greater the movement of nitrogen between different forms, before the amino acid pattern required by the body is achieved, the greater the amount of nonessential nitrogen that needs to be consumed in the diet in order to achieve nitrogen balance. This hierarchy of effectiveness is shown in Table 1, and it can be seen that in relative terms urea is a poor source of nonessential nitrogen when compared with ammonia or nonessential amino acids. The most effective mixture is glutamic acid and glycine, and the addition of glycine will enhance the efficiency with which any other mixture of nonessential amino acids or nonessential nitrogen is utilized [2]. The metabolic demands for glycine are considerable, and it is our experience that glycine is most likely to become the first limiting amino acid on a low-protein diet. We have shown that the urinary excretion of 5-L-oxoproline might be used as a marker for glycine status, with excretion increased when the availability of glycine to metabolism is inadequate. The excretion of 5-L-oxoproline is significantly increased in individuals consuming a low-protein diet, and this can be effectively corrected by the consumption of additional nitrogen, either as protein or as urea [55] (Fig. 5). These data implicate the salvage or urea nitrogen in the colon as being central to nitrogen, amino acid, and protein homeostasis.
Nitrogen Trafficking and Recycling Through the Human Bowel

Table 1. The dietary requirement for amino acids is determined by the pattern of amino acids needed for protein synthesis and other pathways.

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<th>Rank order for intake of total nitrogen</th>
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<tr>
<td>1 Essential amino acids at twice minimum requirements with nonessential amino acids</td>
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<tr>
<td>2 Essential amino acids at minimum requirements with nonessential amino acids</td>
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<tr>
<td>3 Essential amino acids at minimum requirements with nonessential nitrogen</td>
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<table>
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<tr>
<th>Rank order for intake of nonessential nitrogen</th>
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<tbody>
<tr>
<td>1 Nonessential amino acids</td>
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<tr>
<td>2 Glycine and glutamate</td>
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<tr>
<td>3 Glycine and diammonium citrate</td>
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<tr>
<td>4 Glycine and urea</td>
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<tr>
<td>5 Diammonium citrate</td>
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<td>6 Urea</td>
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The relative efficiency with which this demand can be satisfied is determined by the pattern of amino acids and N in the diet. The more metabolic exchange of N to achieve a pattern of amino acids which matches the demand, the higher the total N intake required to achieve N balance.

Fig. 5. The excretion in urine of 5-L-oxoproline has been used as an index of the sufficiency of glycine for metabolism. As the dietary consumption of nitrogen decreases the excretion of 5-L-oxoproline increases, indicating that the ability to synthesize adequate amounts of glycine might be constrained on a low-nitrogen intakes [55].
Conclusions and Implications

The need now is to define the extent of nitrogen movement through the bowel, its qualitative interrelations, its interactions with other macronutrient and micronutrient components of the diet, and the impact of disorders of the bowel and other diseases with a direct relation to protein homeostasis. Because much of the data on the rates of whole body protein degradation have been based upon the assumption that the only source of essential amino acids is from the diet or protein degradation, the demonstration that there may be substantial \textit{de novo} synthesis by the colonic microflora requires that these theoretical models be reevaluated. Furthermore, there is a need to reinterpret how protein turnover might respond to differences in the level of dietary protein or to situations where there is an increased demand for net protein deposition.

The assumption that the small intestine and colon act in series is only partially true. For nitrogen they appear to act in parallel, being responsive to different drivers, and allowing different patterns of metabolic control through the selective partitioning of the movement of nitrogen and nitrogen containing compounds.

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Nitrogen Trafficking and Recycling Through the Human Bowel

Nitrogen Trafficking and Recycling Through the Human Bowel


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**Discussion**

*Dr. Lundholm:* I would like to ask a provocative question. Could everything be a matter of a chemical exchange of nitrogen between or among metabolic compounds, and not a matter of biochemical reactions?

*Dr. Jackson:* For me, metabolic turnover is all about chemical exchange. If it isn’t about chemical exchange, then I don’t know what it is about. When we measure protein turnover, we look at the flow of amino acid into and out of protein, which is a chemical exchange. The important question is, what is the nature of that chemical exchange and does it have any functional significance? I think it does, because if you interfere with it, or if it is absent, then you get problems as a result. If you get problems as a result of interfering with a process of chemical exchange, then for me that has functional relevance. If you are asking whether the movement of $^{15}$N into glutamic acid was simply an exchange reaction and therefore didn’t represent any net movement, I feel it’s a bit
Nitrogen Trafficking and Recycling Through the Human Bowel

like wondering how many angels can dance on the head of a pin. I don’t think that it moves us forward.

Dr. Lundholm: I would like to explain myself further. Working with labeled tritiated water, for example, it is possible to show that some of that label can be incorporated into protein without any enzymes or other biologically active compounds being present. What I meant by ‘chemical exchange’ was a true exchange of molecules in a certain defined structure without any metabolic meaning in terms of entropy. This leads to another question: can it be shown that bacteria can synthesize amino acids from only urea and perhaps some other additional compounds present?

Dr. Jackson: As to your first point, it’s absolutely correct that if you label amino acids with deuterated hydrogen, then deuterated hydrogen can be exchanged at an aminotransferase site, and you haven’t done anything to the amino acid at all. I don’t think that the movement of amino groups falls into that category. I believe that movement of amino groups from one amino acid to another is of a qualitatively different nature. You asked whether the processes I have been talking about could be due to bacterial utilization of urea, and that is exactly the assumption that is made. The assumption is that the bacteria in the colon act as a complex continuous culture and the different classes of bacteria fulfill different biochemical reactions, one for another. So it is not necessarily the same organisms that split the lactose-ureide as split the urea, and it’s not necessarily the same organisms that split the urea as split the amino nitrogen into specific pathways, but together there is a complex ecosystem which serves to produce the material that the ecosystem requires. The reason I talked about bacterial demand and the importance of complex carbohydrates as an energy source for bacterial growth is that if bacteria are to grow and have an energy source, they are going to require a nitrogen source. To find an adequate nitrogen source, they will need to use urea nitrogen.

Dr. Lundholm: Yes, I understood that that was the concept, but if we take colonic bacteria and culture them in the laboratory, could we then show that if we provide them with only urea and perhaps something else they could then synthesize pure biochemically detectable lysine?

Dr. Jackson: Yes.

Dr. Grimble: It’s interesting that you’re talking about what hepatologists have been doing for years when they feed patients with lactulose to try to trap ammonia from within the colon. My question is, why is the process under control? It seems to have all the characteristics of control. Are we looking at control of the urea transporter in the colon in the same way as you see it in the renal tubule?

Dr. Jackson: I don’t know of any data for the human that directly address the control of that transporter in the colon, but the equivalent transporter in the kidney is responsive to dietary protein. We’ve known for years that part of the response to a low protein diet is related to the vasopressin-induced changes in the collecting duct. So at present the argument is seductive, though circumstantial, that on a low-protein diet there is a stimulus to retain urea in the kidney at the level of the collecting duct through the transporter, and at the same time secrete that urea into the colon through the equivalent transporter. The evidence we have from rats and sheep and other animals is in favor of that process taking place.

Dr. Reeds: It’s dangerous to jump from sheep to monogastric species such as humans, because the sheep recycle a lot of the urea before the small intestine rather than after it. Seductive though the idea may be, there’s substantial farm animal literature where people have sought to show that there is nutritional benefit to be derived from introducing amino acids or proteins, or indeed nitrogen, into the colon, but they have by and large been unable to demonstrate anything at all. My question is, what is the evidence that there is amino acid transport from the colonic lumen to the body, because you still require to get the amino acids back in?
Dr. Jackson: I was careful not to extrapolate from sheep to human data. These are questions that we have to find the answer to and that’s what the next program is going to be about. I would like to come back to the question that was asked of Dr. Grimble in relation to the capacity of the small intestine. In my clinical practice, I am most impressed by the ability of some patients with intestinal failure and short bowel to accommodate their digestive/absorptive capability with time. We have more than one example of people who have effectively lost their entire small intestine and have their duodenum connected to their colon but still maintain a normal body weight and body habitus. Therefore I am forced to the conclusion that they have adapted and may have fertilized their colon. It may be that in order for the colon to respond appropriately it requires an appropriate metabolic set of conditions. Thus simply by putting urea into the colon does not necessarily generate that response. We simply don’t understand the nature of the adaptation. There are dipeptide transporters in the large bowel, and the observation that amino acids appear to get across suggests there is a mechanism for this.

Dr. Young: You’ve made the assumption that the synthesis of lysine, for example, is a colonic function, but you haven’t demonstrated that fact unequivocally. We’ve carried out studies with $^{15}$N-ammonium chloride as a possible substrate for lysine in patients with ileostomies and found that they are as capable of making $^{15}$N-labeled lysine as are healthy MIT students. So there’s something going in the upper small intestine that is of significance here.

Dr. Jackson: I think you’re probably quite right. My suspicion is that patients with ileostomies do not have a normal small intestine, equivalent to that in people with an intact bowel. Patients with ileostomies develop a substantial fecal flora in their lower small intestine which appears to take on some of the functional characteristics of a colonic microflora, giving periodicity to stool loss, enabling development of formed stools, and so on; in fact it operates like the colonic microflora in normal individuals. I agree that we haven’t shown conclusively that lysine synthesis was colonic. This is because unfortunately at the time when we placed urea directly in the colon, we didn’t have the ability to trace the fate to lysine. When we have tried to do this noninvasively by using lactose-ureide to deliver urea to the colon, we couldn’t be 100% certain that all of the lactose-ureide was delivered only to the colon and with none being metabolized before it reached the colon. Therefore I have tried to suggest that we are increasingly confident that these things are likely, and I’ve tried to suggest that pinning them down absolutely is going to be a very important challenge.

Dr. Hunter: You’re working on the assumption, as I understand it, that any $^{15}$N-that isn’t excreted in the stools or the urine is retained in the body. I appreciate that you’re excluding anyone with *Helicobacter pylori*, but we know that the gut flora is capable of making volatile compounds which may be excreted on the breath. I wonder if you’ve excluded that as a possible further source of loss of $^{15}$N-urea?

Dr. Jackson: That’s a consideration that has been revisited on a regular basis. The consensus of opinion now is that the amounts involved are too small to be important; similarly with sweat. They are not of the order that would make a substantial dent in the argument.

Dr. Barbul: As a surgeon, I would take issue with your statement that a duodenal-colonic hook-up is sufficient for maintenance of body weight. You do need a piece of small bowel, perhaps 30 cm in the neonate, which then adapts. But with the duodenum and large bowel alone, though there may be some nitrogen uptake, it will not be sufficient to maintain body weight. Where this procedure is done, it is more for maintenance of fluid and electrolyte balance than for nitrogen balance. From clinical experience I can tell you that you do need some small bowel.

Dr. Lundholm: I think we can resolve the dilemma by saying that to determine the length of the intestine is very difficult during surgery.
Nitrogen Trafficking and Recycling Through the Human Bowel

Dr. Bentsen: I can confirm that the peptone gene is expressed in the colon. It’s in the cecum, at least in the rabbit, and that has been published. Second, there is some evidence that after colectomy there is upregulation of some transporters, at least in the ileum, and one of these is the STLT1 gene. Third, I think it’s very difficult to talk about short gut in this context, because so many other factors are altered in short bowel patients. In particular something odd seems to happen to the immune system of the gut when you chop away a big piece of it, and affected children then act very much like children with food intolerance or cow’s milk protein allergy; they do not seem to gain oral tolerance in a normal way and that gives rise to a lot of other changes that we cannot account for.

Dr. Silk: Your concepts are very interesting but can I ask you to look at them the other way around? Let us assume that there are no mechanisms available in the colon to translocate free amino acids and peptides, because that’s what the basic evidence shows at the moment. There isn’t a scrap of evidence that I know of that in man colonic transport systems exist for free amino acids and peptides. I accept the molecular biological evidence of mRNA expression but I know of no evidence. Assume for the moment that the explanation for salvaging nitrogen from the colon is untenable. How would you then interpret your data?

Dr. Jackson: The data show that nitrogen gets from the lumen of the colon into the system. The question is, in what form? The only other way in which this quantity of nitrogen could be moved would be as ammonia, but so far as we are aware, the handling of that quantity of ammonia does not fit in with the way in which the nitrogen is retained. Therefore in the absence of any other rational alternative way for nitrogen to be moved into and retained within the system, one is forced to deduce that it must be being transported. That may be untenable, but Dr. Young, who is a skeptic, has done similar studies and he was surprised to find that label was present in the host in lysine. This either requires there to be metabolic pathways in mammals that we are all completely unaware of, or it requires an ability to move nitrogen from the lower bowel back into the host. That’s where the situation stands.

Dr. Lundholm: Dr. Young, if you infuse an end-label precursor like urea and determine the appearance of the label in lysine, is that the same as saying that lysine is synthesized from urea in a complex integrated organism?

Dr. Young: What it does say is that the 15N- of urea can ultimately appear in the nitrogen moiety of lysine. It says nothing about the route by which that occurred or the site at which it occurred. I believe a lot of this is going on outside the colon and that Dr. Jackson’s assumption that the colon is the site of great things may be a bit overstated.

Dr. Lundholm: The problem I have is that the movement of label from one compartment to another is not necessarily equivalent to a metabolic pathway or system. I could suggest various ways in which label could jump from bacterial compounds into the metabolic pool without taking part in defined metabolic pathways. That is the dilemma we have to confront.