Annu. Rev. Nutr. 1998. 18:385–411

NITROGEN CYCLING IN THE GUT

Malcolm F. Fuller
The Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, United Kingdom; e-mail: mff@rri.sari.ac.uk

Peter J. Reeds
Children's Nutrition Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030; e-mail: preeds@bcm.tmc.edu

KEY WORDS: endogenous, protein, digestion, absorption, amino acids, urea, microflora

ABSTRACT

This review examines the involvement of the gastrointestinal tract in the utilization of nitrogen, the identities of the nitrogenous substances entering and leaving the gut, and the significance of this recycling in the overall nitrogen economy of the body. It is concerned with nonruminant mammals, including man.

CONTENTS

0199-9885/98/0715-0385$08.00

NITROGEN CYCLING VIA THE GUT LUMEN

Because the gastrointestinal tract digests and absorbs dietary protein, the net movement of nitrogenous substances is from the gut lumen into the body. There is, in addition, a simultaneous movement of nitrogenous substances (as well as water and electrolytes) into the lumen. These enter in a variety of digestive secretions: bile, mucins, sloughed cells or cell debris, urea, and other nitrogenous substances, such as glutathione (3, 77). Some, bile for example, can be measured directly, whereas others, notably mucosal secretions and cellular material, can only be estimated.

Much of the nitrogenous matter secreted into the gut lumen is itself subject to digestion and reabsorption, so only a small fraction of the total nitrogen secreted into the lumen is lost in the feces. However, much of what is recovered is in a form other than that in which it entered, because the gastrointestinal microflora meet their own nutritional requirements through the degradation of both food-derived and endogenous substances. Because these organisms possess enzyme activities their hosts do not, they can utilize substrates, notably complex carbohydrates, that are resistant to mammalian digestive enzymes. These substrates include dietary nonstarch polysaccharides as well as glycoconjugates synthesized by the host. The endogenous nitrogen sources also include urea that enters the lumen by both secretion and diffusion and is degraded there by bacterial urease, making additional nitrogen available for microbial growth. Urea nitrogen, along with other nitrogen sources, may therefore be incorporated, through the de novo synthesis of amino acids, into microbial protein that can then be digested and absorbed and thus contribute to the amino acid supply of the organism. The provision of amino acids to the host by this route is of greatest importance in ruminant species that can meet their amino acid needs for maintenance and some degree of productivity from nonprotein nitrogen sources. We discuss the growing evidence that favors the idea that this route of nitrogen metabolism is significant in the amino acid nutrition of nonruminant species.

Nitrogenous Secretions into the Gut

To build a complete picture of nitrogen movement into and out of the gut, it is necessary to combine data from different experiments. To be compatible, however, these experiments must be done on the same species at the same stage of development. The most coherent body of data that fulfils these criteria is from experiments with growing pigs.

Direct measurements of specific secretions have been made with cannulas placed at various sites in the porcine gut. The maximum flow of saliva in pigs 30–40 kg was measured as 500 g/day (17). From this, Juste (54) calculated that the saliva might contribute 400 mg of nitrogen daily. Using a sampling-replacement method, Corring et al (19) then estimated that total bile nitrogen

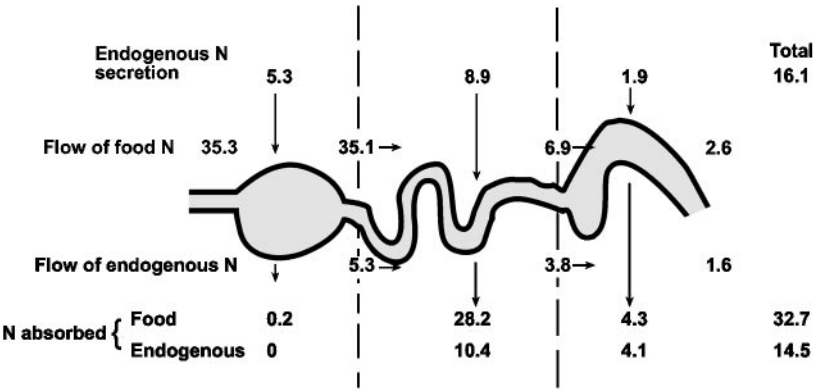

Figure 1 Flow, secretion, and absorption of N (grams/day) in three sections of the digestive tract of 30-kg pigs estimated by ^{15}N labeling and digesta exchange (57, 58).

flow in pigs is 1.7 g/day and that pancreatic secretion supplies approximately 1.9 g/day to the lumen of the upper small intestine. These three components together account for most of the endogenous N flow through the proximal duodenum (Figure 1).

The Contribution of Endogenous Sources to the Nitrogen of Digesta

Direct measurement of secretory outflow from individual organs does not address three questions that are crucial to describing the dynamics of nitrogen cycling in the gut. What is the total secretion of protein and other nitrogenous substances from the mucosa? What fraction of the nitrogen flowing down the gut is of endogenous origin? What fraction is reabsorbed in the small intestine and what fraction enters the large bowel? To answer these questions requires methods that distinguish endogenous protein in the digesta from undigested dietary protein. Though this is not possible by any direct separation, it can be done indirectly by one of a variety of methods, the assumptions and limitations of which have important implications for the interpretation of the results. The most important of these methods are described below.

FEEDING PROTEIN-FREE DIETS The most direct approach to estimating endogenous protein flow is to feed test animals protein-free diets, when it can safely be assumed that all nitrogen in digesta is of endogenous origin. This involves the assumption that neither the rate nor the amino acid composition of the endogenous secretions is affected by ingestion of protein. This assumption is difficult to prove experimentally, but there are some relevant observations. Using pigs with cannulated pancreatic ducts, Corring et al (18) found that pancreatic nitrogen

output was no different on a protein-free and on a normal (14%) protein diet. Similarly, Zebrowska et al (114) reported that although the total volume of pancreatic juice in pigs given a cereal-based diet was twice as great as when they had a synthetic diet, the daily pancreatic output of nitrogen was similar (2.0 and 2.1 g). On the other hand, indications are that the amino acid composition of the endogenous outflow is different under different conditions. de Lange et al (21) found that the protein status of pigs influenced the composition of their endogenous secretions, notably the appearance of proline and glycine, a result confirmed by Leterme et al (60).

Another method, which is often claimed to circumvent the argument that protein-free diets may alter endogenous flow, is to feed test animals diets with graded concentrations of protein and then extrapolate the linear regression of digesta nitrogen flow on nitrogen intake to zero. However, this approach also involves the assumption that endogenous secretion is unaffected by protein intake. Clearly, methods are needed that distinguish between endogenous protein and the undigested dietary protein of a normal diet.

ESTIMATING ENDOGENOUS AND EXOGENOUS CONTRIBUTIONS TO DIGESTA FROM AMINO ACID PATTERNS By feeding test animals proteins that have a distinctly different amino acid composition from that of their endogenous secretions (59, 80), it is possible to estimate the relative contributions of the two sources by comparing the amino acid composition of the digesta with that of the dietary protein and of digesta collected in the postabsorptive state or during protein-free feeding (Table 1). From these data, simultaneous equations are used to calculate what proportion of each amino acid is contributed by the diet and what is from endogenous sources. These values may then be combined in a multiple regression to estimate the average proportion of the digesta nitrogen derived from endogenous sources. In its most extreme form, the method uses dietary proteins such as zein (15) or gelatin (N de Roos, I Grant, D Brown, MF Fuller, unpublished data), devoid or almost devoid of a single amino acid so that any of that amino acid in digesta indicates its endogenous origin. The method does, however, require the assumption that undigested dietary protein has the same amino acid composition as the protein ingested—in other words, that there is no selective digestion or absorption. Baglieri et al (4, 5) found that the contributions of some amino acids (e.g. glutamate, methionine, leucine, tyrosine, histidine) to the jejunal digesta of humans consuming either casein or soya milk protein were proportionately lower than the contributions of those amino acids to either the diet or the endogenous outflow. This suggests that the removal of these amino acids from the small intestinal lumen is more efficient than the removal of others. However, it also assumes a constant recycling of endogenously secreted protein (89, 90), and this may not be so (100).

Table 1 The amino acid composition of jejunal and ileal digesta samples taken from human subjects in the fasted state or while receiving a protein-free diet and by a regression approach in protein-fed pigs

	Amino acid composition (mg/g of total amino acids)				
	Human		Pig		
Amino acid	Jejunal postabsorptive[a]	Ileal protein-free[b]	Ileal protein-free[c]	Ileal protein-free + i.v. amino acids[c]	Ileal intercept method[d]
Thr	66	88	50	65	63
Lys	52	47	34	44	43
Met	14	12	12	16	12
Leu	76	70	42	55	61
Ileu	40	38	25	33	40
Val	62	46	36	50	50
Phe	63	42	49	58	34
Arg	47	75	34	33	60
His	26	42	14	17	21
Tyr	42	42	26	32	32
Ala	42	46	40	46	50
Ser	69	68	46	57	64
Gly	56	ND	78	66	106
Glut + Gln	96	112	75	94	87
Asp + Asn	82	101	67	81	80

[a]From Baglieri et al (4) and Mahé et al (66).
[b]From Fuller et al (34).
[c]i.v., Intravenous. From de Lange et al (21).
[d]From Fan & Sauer (29).

In an interesting variant of this approach, Fan et al (30) and Fan & Sauer (29) showed a strong linear relationship in pigs between protein intake and the apparent ileal digestibility of amino acids. The closeness of the relationship suggests that variations in protein intake had little impact on the amino acid composition of the secretions.

The results of such approaches in studies of jejunal protein digestibility in humans and of ileal protein digestibility in pigs suggest that the "true" digestibility of most proteins is >85% and that endogenous sources contribute between 35% and 60% of total jejunal nitrogen flow.

FEEDING PEPTIDES TO DISTINGUISH ENDOGENOUS FROM EXOGENOUS NITROGEN IN DIGESTA By feeding to test animals a protein hydrolysate or a mixture of peptides, assumed to be fully absorbed before reaching the end of the ileum, all the protein in the digesta at the end of the ileum and beyond is assumed to be endogenous (14). This allows measurements of endogenous nitrogen flow to be made while nitrogen intake is varied. Using this method with small pigs (8 kg) in combination with an isotopic method (see below), Schulze et al (83)

arrived at an estimate (1.4 g of N/day) that was slightly but not significantly higher than the estimate arrived at simultaneously by ^{15}N dilution (1.2 g/day).

FEEDING PROTEINS LABELED WITH HOMOARGININE By treating food proteins with O-methylisourea, lysine residues are converted to homoarginine (44). The two assumptions are that homoarginine is absorbed to the same extent as lysine and thus serves as a marker of undigested dietary protein and that the modified protein is digested to the same extent as the original. The last, however, does not appear to be true (24).

USING LABELED DIETS TO MEASURE THE CONTRIBUTION OF DIETARY PROTEIN TO DIGESTA NITROGEN There is an increasing literature that describes the feeding to test animals of proteins labeled with either ^{15}N (41, 67, 79) or ^{13}C (8, 9, 13, 92) to study protein digestion and amino acid absorption. In studies of digestion, the intestinal flow of total nitrogen and carbon is compared with that of ^{15}N or ^{13}C, and the contribution of endogenous sources is calculated from the changes in isotopic enrichment in the intestinal lumen. Using this method to determine "real" digestibility leads to the conclusion that many dietary proteins are efficiently digested, with casein being nearly 60% digested within the proximal jejunum. Combined with further analysis of labeled peptide fragments, this is a useful approach to the study of intestinal protein dynamics (66, 79). This method has also been used effectively in the study of the importance of gastric emptying in the regulation of protein digestion (40, 65, 66).

However, when used to quantify the flow of endogenous protein, this method assumes that over the course of the measurements all endogenous secretions are unlabeled. Leterme et al (61) showed in pigs that within 50 min of consumption of a ^{15}N-labeled diet, tracer appears in pancreatic secretions, and within 4 hours of consumption, it appears in terminal ileal mucins. These results make it likely that the labeled-diet method systematically underestimates the endogenous secretion rate and thereby the true extent of dietary protein digestion.

LABELING ENDOGENOUS PROTEIN BY PROLONGED INTRAVENOUS ^{15}N-TRACER INFUSIONS The other isotopic approach used on both pigs (23, 62, 63, 89, 90) and humans (38, 39, 86) is to label the host organism. This method, first applied systematically by Souffrant et al (89), involves the prolonged (>5 days) administration of a ^{15}N-labeled amino acid with the aim of reaching isotopic equilibrium in the protein secreted into the intestine. Once this condition is attained, the isotopic enrichment in the ileal digesta (23) or in the feces (86) (Figure 2) should, in theory, give a direct measure of the fractional contribution endogenous amino acid nitrogen makes to the total nitrogen in the digesta. If total ileal (or fecal) nitrogen flow is then measured, the absolute amounts of endogenous (labeled) and exogenous (unlabeled dietary) nitrogen passing

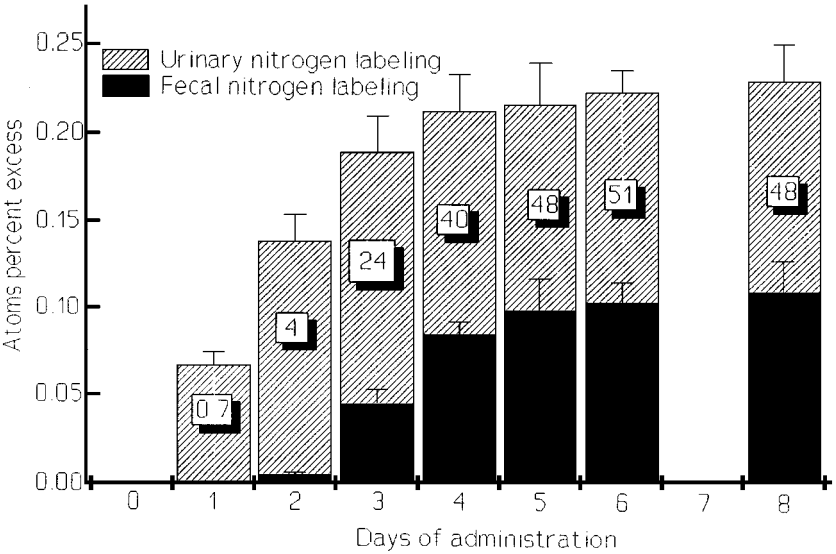

Figure 2 Time course of urinary and fecal ^{15}N-labeling in infants receiving a constant oral input of [^{15}N]glycine (86). Numbers within the columns are percent endogenous contribution to fecal nitrogen.

the terminal ileum can be calculated. de Lange et al (23) found that ileal endogenous nitrogen outflow was approximately 25 g/day in 60-kg pigs and that endogenous nitrogen contributed between 50% (canola diet) and 82% (wheat diet) of the total ileal nitrogen flow. Shulman et al (86) similarly obtained an estimate (51%) of the contribution of endogenous nitrogen to fecal nitrogen in formula-fed infants.

Although ^{15}N-dilution is a valuable technique, applications have suffered from the use of a single amino acid tracer, in particular [^{15}N]leucine, and from inappropriate choices of the fractions analyzed (discussed in 62, 63). Leucine was originally chosen as a tracer on the assumption that it would freely label other amino acids via transamination, but it has been shown (22, 62) that the isotopic enrichment of other amino acids, even of isoleucine and valine, was less than that of the tracer leucine. Thus, measurements of total ^{15}N-, [^{15}N]leucine, and other ^{15}N-labeled amino acids isolated from the total nitrogen or protein amino acid fractions gave different estimates of the contribution of endogenous proteins and, hence, of absolute protein digestion (22, 62, 63). The problem is compounded further by the fact that measurements of the labeling of mucosal protein have only rarely been made. Lien et al (62) measured the isotopic enrichment of crude isolates of intestinal mucin in ^{15}N-labeled pigs, and these

results revealed the interesting phenomenon that isotopic equilibrium between the plasma and mucin pools of isoleucine and valine (i.e. amino acid labeling derived from [^{15}N]leucine label) was achieved while the [^{15}N]leucine isolated from the mucin was of lower isotopic enrichment than the plasma leucine pool. The results also highlighted the potentially important contribution of bacterial nitrogen (which had a relatively low isotopic enrichment, see below) to the ileal digesta.

Just as the use of labeled diets described above leads to the rapid labeling of endogenous secretions, so—in this approach—unlabeled dietary amino acids dilute the enrichment of endogenous secretions. Both lead to an underestimate of the endogenous contribution to digesta nitrogen. However, despite these caveats the results obtained with this technique present the most coherent body of data on the quantitative contribution of the endogenous secretions.

Using this approach with pigs that had implanted cannulas, Krawielitzki et al (57, 58) exchanged the digesta (Figure 3) between a pig labeled with [^{15}N]leucine and two not labeled. Their results (Figures 1 and 3) suggest that the secretion of endogenous "protein" in 30-kg pigs is approximately 16 g/day, corresponding to approximately 15% of total body protein turnover (78). From a similar experiment with 50-kg pigs given a lower protein intake, Souffrant et al (90) estimated that total endogenous secretion was approximately 11 g/day. In both experiments the resorption of endogenous nitrogen was estimated at close to 90%.

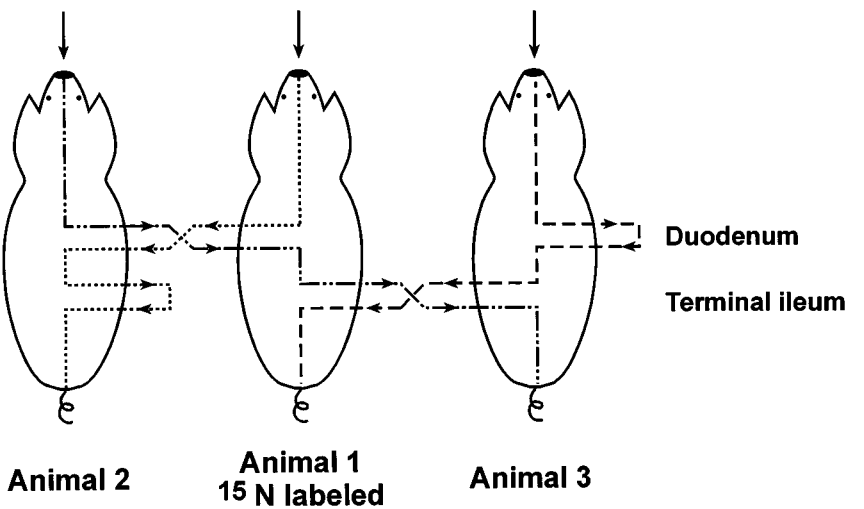

Figure 3 Scheme for digesta exchange in pigs (57, 58, 99).

Table 2 ^{15}N enrichment of TCA-soluble and TCA-precipitable fractions or digesta in various segments of the gastrointestinal tract of pigs at the end of an i.v. infusion of [^{15}N]urea[a]

Segment	TCA-precipitable fraction of digesta	TCA-soluble fraction of digesta
Stomach	0.01	0.16
Small intestine	0.18	0.71
Cecum	0.04	0.18
Colon	0.02	0.10
Rectum	0.00	0.02

[a]TCA, Trichloroacetic acid; i.v., intravenous. Results show atoms percent ^{15}N excess. From Reference 7.

Urea

Part of the endogenous nitrogen entering the gut is as urea. Although there is no ready means of estimating directly the amount of urea entering the gut lumen, the amount hydrolyzed there, and thus available for further metabolism (either by the microflora or the host), can be estimated as the difference between the rates of urea synthesis and urinary urea excretion, with the assumption that the only ureolytic activity is microbial. The rate of urea recycling depends on two factors: the rate of its diffusion and secretion into the lumen, and the rate of its hydrolysis by wall-adherent microbes.

Urea is secreted into all parts of the digestive tract, but secretion is more active in the small intestine than in the stomach or large intestine (7, 73) (Table 2). From the results of their experiments with [^{15}N]urea infusion, Bergner et al (7) suggested that the main route of entry into the small intestine is via pancreatic juice, via bile, or both and that it amounted to 2.4 g/day in 34-kg pigs. Urea secretion also appears to be higher in the proximal small bowel than in the ileum, although there is a progressive incorporation of urea nitrogen into trichloroacetic acid–precipitable nitrogen as the digesta progress through the small intestine. Thus, in the study by Bergner et al (7), only 5% of the duodenal ^{15}N was precipitated by trichloroacetic acid compared with 43% in the ileal digesta. These data also showed that the protein-bound digesta nitrogen had a higher isotopic enrichment than mucosal protein nitrogen (Table 3), which suggests that the majority of the fixation of the ^{15}N of urea had been by microorganisms. The relatively small uptake of urea by the large intestine is confirmed by other experiments. Malmlöf & Simoes Nunes (68) perfused loops of jejunum and colon in pigs. Infusion of urea into the jugular vein led to fivefold higher concentrations of both urea and ammonia in the jejunal perfusate than in the colonic loop. In pigs, little ileal urea appears to exit to the cecum (73), and in humans, Wrong

Table 3 ^{15}N enrichment of the TCA-precipitable fraction of mucosal protein from various parts of the gastrointestinal tract of the pigs at the end of an i.v. infusion of [^{15}N]urea[a]

Tissue	Atom% ^{15}N excess
Duodenal mucosa	0.04
Jejunal mucosa	0.02
Ileal mucosa	0.03

[a]TCA, Trichloroacetic acid; i.v., intravenous. From Reference 7.

et al (106) found that fecal ammonia and fecal bacterial nitrogen had only 8% of the ^{15}N enrichment of plasma urea. This suggests that systemic urea is a minor substrate for the growth of a major portion of the colonic bacteria.

Despite this, there is evidence that a significant quantity of urea is hydrolyzed. In many studies of both animals and humans, that value is approximately 20–25% of urea production (28, 35, 51, 64, 101), although in some studies (e.g. 10) values as high as 40% of total urea production have been noted. There is no consensus on whether this urea cycling is obligatory and fixed in magnitude (see 27 for discussion), whether it is simply proportional to the circulating urea concentrations (as implied by the results in 35), or—as argued by Jackson et al (e.g. 31, 49, 70)—whether it is regulated and sensitive to the balance between amino acid demands and protein supply.

The fate of urea entering the small intestine is not entirely clear, but the degree to which, and the forms in which, urea nitrogen is returned to the body have an important bearing on the hypothesis of Jackson et al regarding the nutritional significance of urea cycling. The fraction of urea that recycles immediately to urea can be examined by administering [^{15}N,^{15}N]urea and measuring the reappearance of the label in [^{14}N,^{15}N]urea (50, 64). Here again there is no agreement. Jackson suggests that approximately 80% of the hydrolyzed urea nitrogen is retained in amino acids within the body, whereas earlier data (64) suggested that $>75\%$ returns rapidly to the urea pool. In fact, Long's kinetic data (64) showed virtually equal return of the carbon and nitrogen moieties of urea to the urea pool, evidence in favor of there being a metabolically significant rate of urea resynthesis at the site of hydrolysis (i.e. by the mucosal cells). This idea is compatible with recent observations of readily measurable urea synthetic activity in porcine small intestinal enterocytes (107). Resynthesis of urea within gastrointestinal tissue would help to reconcile the apparent discrepancy between measurements of urea hydrolysis derived from isotope dilution studies and measurements of urea uptake by the gut from mass balance studies.

Despite these uncertainties as to the fate of its nitrogen, a substantial proportion of urea synthesized in the body is hydrolyzed and this can occur only

in the gut. Whether this urea/NH_3 cycle is of nutritional benefit is discussed below.

UTILIZATION OF AMINO ACIDS SYNTHESIZED BY GASTROINTESTINAL FLORA

Although microbial protein makes an important contribution to the amino acid needs of ruminant animals, it has been considered to be of no nutritional significance to nonruminants. This view was based on the recognition that the greatest microbial population is concentrated in the cecum and colon whereas the major site of amino acid absorption is the small intestine. This is true, but the small intestine is not the only part of the gut capable of amino acid absorption nor is microbial activity confined to the large intestine. It is therefore important to know the extent of microbial activity in the upper gastrointestinal tract and the capacity of the large bowel to absorb amino acids.

Amino Acid Absorption from the Large Intestine

Active absorption of amino acids by the large intestine was demonstrated in newborn pigs (87) but the ability is lost progressively with age (52, 84). Studies with germ-free animals suggest that the age-related decline in cecal amino acid absorption may be associated with microbial colonization (46). Furthermore, there is evidence for selectivity in cecal amino acid transport. Olszewski & Buraczewski (74) studied adult pigs with sterilized ceca. They found that the greatest absorption was for substituted amino acids (asp, ser, thr, tyr, arg, his, lys). In this context it is perhaps significant that many of the experiments failing to show amino acid absorption from the mature large intestine (11, 52) used the amino acids that Olszewski & Buraczewski (74) found not to be absorbed.

The large intestine evidently has the physiological capacity to absorb some amino acids, but this does not necessarily mean that the process is of nutritional significance. For one thing, the competition between microbe and host for free amino acids is probably generally in favor of the microbe. Nevertheless, the potential contribution of amino acids absorbed in the large intestine toward meeting an animal's requirements has been explored by infusing protein or amino acids directly into the terminal ileum or cecum of fistulated animals. A summary of such experiments is given in Table 4.

These experiments show that protein infused into the large intestine is digested: There was no increase in fecal nitrogen excretion. However, most of the experiments showed no significant improvement in nitrogen balance, and it was concluded that the large intestine made no significant contribution to the amino acid economy of the animal [except Gargallo & Zimmerman (37)]. Yet the tendency is almost always toward improved nitrogen balance, which suggests that with greater experimental precision, statistically significant differences

Table 4 The effects on nitrogen balance of giving protein or amino acids orally or by infusion into the large intestine of pigs

Infusion	Oral nitrogen	Infused nitrogen	Urinary nitrogen	Fecal nitrogen	Nitrogen balance	Reference
Protein						
Casein	1.6	0	3.8	2.0	−4.2	112
	1.6	17.0	18.9	3.0	−3.2	
Casein	1.0	0	3.5	1.9	−4.4	112
	1.0	16.6	17.3	1.5	−1.3	
Casein	28.1	0	13.6	8.6	5.9	113
	28.1	15.5	26.5	8.2	8.9	
Casein	51.6	0	16.8	7.9	26.9	37
	52.9	8.2	20.4	8.3	32.4	
Amino acids						
Basal	37.4	0	—	—	6.4	108
+ lysine	43.6	1.7	—	—	6.6	
Basal	50.6	0	—	—	17.9	108
+ lysine	50.6	0.3	—	—	19.4	
Basal	44.5	0	—	—	10.9	108
+ lysine	44.5	0.4	—	—	10.7	

might have been reported and more positive conclusions drawn. The identity of the substances absorbed may still be questioned because no nutritional benefit was seen when a single amino acid was infused into the distal ileum or cecum of an animal given a diet deficient in that amino acid (Table 4). Undoubtedly nitrogen is absorbed from the large intestine and is incorporated into body protein, as Heine et al (45) demonstrated in infants by infusing ^{15}N-labeled yeast protein into the colon: Most of the ^{15}N was retained in body protein. Although often taken as proof that intact amino acids are absorbed in the large intestine, evidence from animal experiments suggests that the protein is first degraded to ammonia, which is absorbed, and then labels amino acids in the body. Any small improvements in nitrogen balance resulting from the infusion of protein or amino acids into the large intestine could be due to the absorption of nitrogen as ammonia and its utilization as nonspecific nitrogen. This of course assumes that nonspecific nitrogen itself can be of nutritional benefit.

The Utilization of Nonspecific Nitrogen

The provision of nonspecific nitrogen can improve nitrogen balance under conditions of low-protein feeding (48, 55, 56). The metabolic basis of these observations is not well understood but could include (*a*) balancing the relative quantities of dispensable and indispensable amino acids, (*b*) shifting the balance

of metabolism of the keto acids of the indispensable amino acids toward reamination and away from oxidative metabolism, and (*c*) increasing the supply of all amino acids by stimulating their synthesis by the gastrointestinal microflora. This last possibility presupposes that amino acids synthesized by the intestinal microbes are absorbed and utilized by the host, a supposition examined in detail below.

Regarding the first possibility, nonspecific nitrogen can be incorporated into amino acids (2, 71, 75). After administration of [^{15}N]urea or ^{15}N-labeled ammonium salts, plasma amino acids become labeled to varying enrichments. The most highly labeled are those amino acids (glutamate, glutamine, aspartate, and alanine) that are transamination products of ketoacids generated from glycolysis and the tricarboxylic acid cycle. Serine and glycine are also readily labeled from ammonia, and arginine is particularly highly enriched with ^{15}N when oral ammonia is administered (75). The branched-chain amino acids form a second group, labeled to approximately half the enrichment of the first group, and still lower isotopic enrichments are found in phenylalanine and tyrosine. The lowest enrichments of all are in lysine and threonine. The pattern of amino acid labeling is similar when the ^{15}N is given as urea or ammonia, though the enrichments of plasma amino acids are higher when ammonia is given (71). This suggests that urea may be utilized only after it has been hydrolyzed and its nitrogen has been recycled through the body.

Although transamination accounts for the labeling of most amino acids, it cannot account for the labeling of lysine, which does not undergo transamination (82, 96). The ability of threonine to acquire ^{15}N by transamination is less clear. It is a poor substrate for transamination (6), but its complete inability to transaminate does not seem to have been as rigorously established as that of lysine.

Given that lysine does not transaminate, the appearance, of labeled lysine in the plasma and tissues after giving [^{15}N]urea or other simple nitrogen compounds is presumptive evidence that the labeled lysine (along with other amino acids) was synthesized by gastrointestinal microflora and subsequently absorbed by the host. This raises the possibility that the amino acid needs of nonruminants may not be met exclusively from the diet but may be met in part by the de novo synthesis of the gut flora, a symbiotic relationship like that in ruminants. As it would be expected that amino acids synthesized by the microflora of the small intestine would be readily available to the host, the distribution of microbial activity along the gastrointestinal tract becomes an important consideration.

The Distribution of Microbial Activity in the Nonruminant Intestine

Microorganisms colonize all parts of the gastrointestinal tract but are not uniformly distributed in numbers, species, or metabolic activity (16, 43, 53). The

highest concentrations of organisms, in terms of total counts per milliliter of digesta, are found in the cecum and colon, but other parts of the gut may harbor populations of organisms that are significant in terms of the nature and intensity of their metabolic activity. For example, Jensen (53) showed that in healthy pigs the metabolic activity of the gut flora (measured as ATP concentration and adenylate energy charge) was as high in the distal third of the ileum as it was in the cecum, and the total activity of the small intestine was as great as that of the large intestine. Even in the stomach, lactobacilli and yeasts may be present in significant numbers (81). Ureolytic activity is not expressed by normal gastric flora but is a feature of infection by *Helicobacter*.

The Utilization of Microbial Protein

For amino acids synthesized by the gut microflora to be utilized by the host they must be (*a*) produced in segments of the gut in which amino acid absorption occurs or proximal to such regions, or (*b*) recycled by reverse peristalsis or by coprophagy. Obviously there are marked differences between species in this respect. The rabbit is a nonruminant species that practices coprophagy. In addition, however, the separation of the hard and soft feces in rabbits is accompanied by retrograde flow of cecal contents, so both this and coprophagy may contribute. Torrallardona et al (97) showed that in rats, coprophagy is the exclusive mechanism for the utilization of microbial amino acids. In these experiments, growing rats were given a diet that included [^{15}N]ammonium chloride. In germ-free rats there was essentially no ^{15}N enrichment of body lysine, whereas in rats with a conventional gut flora there was a substantial [^{15}N]lysine enrichment in the whole body, and even higher enrichments in the plasma and liver (Table 5). In rats denied access to their feces, the [^{15}N]lysine enrichment in body protein was no more than in the germ-free animals (Table 6). The daily absorption of microbial lysine (Abs, milligrams per day) was estimated from the equation

$$\text{Abs} = [\text{AA(body)}/n] \cdot [\text{APE(body)}/\text{APE(microbial protein)}],$$

Table 5 The ^{15}N labeling of lysine in rats given [^{15}N]ammonium chloride[a]

Determinant	Enrichment	SEM
Plasma	0.0219	0.00109
Liver	0.0314	0.00047
Whole body	0.0076	0.00049

[a]Results show atoms percent excess. SEM, Standard error of the mean. From Reference 95.

Table 6 [^{15}N]lysine enrichment of conventional rats allowed or denied coprophagy and of germ-free rats[a]

Determinants	Experiment	[^{15}N]lysine enrichment	SEM
Conventional rats			
Allowed coprophagy	1	0.0066	0.00047
	2	0.0066	0.00084
Denied coprophagy	2	0.0003	0.00053
Germ-free rats	1	0.0004	0.00010

[a]In each experiment, the rats were given a low-protein diet supplemented with fermentable carbohydrate and [^{15}N]ammonium chloride. (From References 96, 97.) Results show atoms percent excess. SEM, Standard error of the mean.

where AA(body) is the total weight of lysine in the body, *n* is the number of days of labeling, APE(body) is the ^{15}N enrichment of lysine in the whole body, and APE(microbial protein) is the ^{15}N enrichment of lysine in the microbial fraction of the digesta (in this case feces because it was established that microbial amino acid absorption depended entirely on coprophagy). The estimated absorption of lysine from coprophagy in the conventional rats was estimated to be 23 mg/kg$^{0.75}$ · day, corresponding to about two thirds of their dietary lysine requirement for maintenance. This does not alter traditional estimates of dietary amino acid requirements that have been made by dose-response experiments, but it does mean that the animal's metabolic requirements are not the same as its dietary requirements but substantially higher. This has implications for the discrepancy between estimates of human amino acid requirements made by the nitrogen balance technique and those made from measurements of amino acid oxidation (32).

In experiments of similar design, Torrallardona et al (98) kept young pigs in metabolism cages to prevent coprophagy. Like the rats, they were given a diet that included [^{15}N]ammonium chloride, but in order to examine the uptake of amino acids other than lysine synthesized by the gut flora, their diet also included [U-^{14}C]polyglucose, a substrate resistant to mammalian digestive enzymes but fermentable by the gut microflora. After 10 days on these diets the pigs were killed and the ^{14}C-specific radioactivity (SRA) of indispensable amino acids in enrichments of their carcasses (without the digestive tract, to avoid any possible contamination by digesta) was determined. Radioactivity was found in all the amino acids examined (Table 7), confirming that the phenomenon was not peculiar to lysine.

Because the pigs were given both ^{14}C and ^{15}N labels it was possible to compare the two estimates of microbial lysine uptake. Using the equation above and taking the microbial fraction of digesta from the distal ileum to be the source of the absorbed amino acids, the daily lysine absorption by the pigs was

Table 7 ^{14}C-specific acidity (SRA) in amino acids from the carcasses of pigs given [^{14}C]polyglucose in their diet for 10 days[a]

Amino acid	SRA	SEM
Valine	473	66.5
Isoleucine	290	26.8
Leucine	201	42.6
Tyrosine	351	25.4
Phenylalanine	193	18.4
Histidine	729	107.1
Lysine	352	40.5

[a]SEM, Standard error of the mean. Results show dpm/nanomole. From Reference 99.

estimated from ^{14}C data to be 804 mg/day and from ^{15}N data to be 1220 mg/day. These values are two or three times the animal's dietary lysine requirements for maintenance (33), but because young pigs have high amino acid requirements for growth, they are about one tenth of the total requirement. Those estimates are based on the assumption that absorption of microbial amino acids occurs in the ileum, but if it is assumed to occur in the large intestine and the enrichment (or SRA) of the microbial fraction of cecal digesta is used in the calculation, the denominator in the equation being greater, much lower estimates of absorption result. So to answer the question of whether de novo synthesis of amino acids and their absorption make a significant contribution to meeting the requirements of the host, it is necessary to establish where such absorption occurs and what the microbial amino acid labeling at the site of absorption is. Recent evidence points to the importance of the small intestine. In the experiment by Torrallardona et al (99), ileal digesta from pigs given a diet supplemented with $^{15}NH_4Cl$ was quantitatively infused into the cecum of similar pigs given an unlabeled diet (see Figure 3). The [^{15}N]lysine enrichment in the plasma of the pigs given the labeled diet was three quarters that measured in intact pigs given the labeled diet, whereas that of pigs receiving labeled digesta per cecum was only one quarter that of the intact pigs.

It is also crucial to establish whether de novo amino acid synthesis by the gut flora occurs at the expense of amino acids (whether from the diet or endogenous sources) that, had they not been metabolized by the microflora, would have been absorbed intact anyway. If so, the intervention of the microflora would represent simply an additional step in the utilization of amino acids by the host, without necessarily any net gain in the mass of amino acid available for postabsorptive metabolism. These considerations make it essential to establish not only the site of absorption, but also the identity of the nitrogenous precursor(s) of microbial amino acids.

The contribution of microbial amino acids to meeting requirements could be far more important to the adult human, whose needs are simply to maintain protein homeostasis, than to the growing pig, with its high requirements for body protein accretion. However, the gastrointestinal microflora of the pig are more abundant than those of man, and it is not safe to assume that the rate of de novo amino acid synthesis in man is as great as that in a pig of similar size. Early experiments (36, 42, 85, 93) showed clearly that the same phenomenon occurs in humans, with label appearing in the indispensable amino acids, including lysine, but only recently have experiments been begun to estimate its quantitative contribution to meeting amino acid requirements. Adult human subjects given [^{15}N]ammonium chloride have also shown enrichment of [^{15}N]lysine in the body (72) (MF Fuller, C Garnham, CI Harris, E Milne, G Calder, I Grant, MHN Golden, unpublished observations; CC Metges, KJ Petzke, AE El-Khoury, S Bedri, L Henneman, I Grant, MF Fuller, VR Young, unpublished observations). In these experiments, because of the difficulty of estimating whole-body [^{15}N]lysine enrichment, amino acid absorption was estimated from the plasma flux and enrichment of lysine. But the same difficulty arises as in the animal experiments, namely the need to establish the enrichment of microbial amino acids at the site(s) of their absorption. This was addressed by comparing ileostomates with intact subjects (MF Fuller, C Garnham, CI Harris, E Milne, G Calder, I Grant, MHN Golden, unpublished observations; CC Metges, KJ Petzke, AE El-Khoury, S Bedri, L Henneman, I Grant, MF Fuller, VR Young, unpublished observations). In both experiments there was significant ^{15}N enrichment in the plasma lysine of ileostomates, but not as high as in the intact subjects. Given that the ileal microflora of ileostomates are known to be more prolific than in intact subjects, these results, coupled with those obtained with pigs, suggest that the large intestine plays a role in the process, though whether by absorbing microbial amino acids or merely by returning labeled ammonia to be recycled remains to be established. In addition, the nutritional significance of these observations depends on identifying the immediate nitrogen source for microbial amino acid synthesis.

The Source of Nitrogen Utilized by the Gastrointestinal Microflora for De Novo Amino Acid Synthesis

Because of the rapid and diverse exchanges of nitrogenous substances between luminal contents, microbes, and host, it is technically difficult to establish definitively the sources of nitrogen used by the gastrointestinal flora for amino acid synthesis, especially if the investigation is confined to whatever subpopulation is responsible for producing the amino acids subsequently absorbed by the host. Nevertheless, some clues and indirect evidence emerge.

One feature of the experiments with rats and pigs described above is the linear increase in the labeling of amino acids in feces over the 10-day experiment. If the

gut flora were utilizing ammonium nitrogen directly, a plateau in ^{15}N enrichment should be reached in fewer than 10 days. This points to the existence of a much larger nitrogen pool, which is supplied by the dietary label and which does not reach equilibrium labeling within the 10 days. Not only ^{15}N enrichment but ^{14}C specific activity increased linearly with time. This suggests that the tracer is recycled through a pool supplied from the luminal contents and returns via endogenous secretions. As pointed out, endogenous secretions are rapidly labeled from dietary ^{15}N-labeled proteins. This pool could also include the tissue of the gut itself, especially the villous enterocytes, which, as described below, derive their amino acids predominantly from luminal rather than systemic sources. It could also include microbial cells that in turn become a source of nutrients for other microorganisms.

In experiments by Metges et al (72), the ^{15}N labeling of plasma lysine in adult human subjects was three times higher after administration of [^{15}N]ammonium chloride than after administration of [^{15}N]urea. Even allowing for the greater retention of the ammonium label, a twofold difference remained. Because urea nitrogen is available for further metabolic utilization by either microbes or host only after the urea is hydrolyzed, this might point to the importance of microbial amino acid synthesis in the upper digestive tract by organisms lacking urease. Alternatively, it may suggest that neither [^{15}N]urea-N nor [^{15}N]ammonium-N is utilized directly by the microflora for amino acid synthesis but only after the label has been recycled through the nonessential amino-N pools and returned to the gut in endogenous secretions. This would be consistent with the ^{15}N enrichment of fecal protein after administration of urea despite the lack of evidence for urea entry into the large intestine.

NITROGEN CYCLING BY GASTROINTESTINAL TISSUE

Most of the available information on nitrogen recycling considers the gut as a whole; that is, the measurements reflect the combined activities of the luminal microflora and of the gastrointestinal tissue, which itself comprises a number of different cell populations. Not all the nitrogen taken up by the gut from the arterial circulation enters the lumen. The gut is an organ with vigorous metabolic activity, taking up amino acids and other nitrogenous substances from the blood. Indeed, the gut, as an organ, has one of the highest rates of protein synthesis of any tissue in the body (102). This is to be expected given the large number of rapidly dividing cells, especially those of the mucosa and the gut-associated lymphoid tissue and its high secretory activity. However, the use of amino acids for protein synthesis does not necessarily involve a net loss from the body, as the amino acids subsequently released by protein breakdown are

available for reutilization. Even so, the utilization by gut tissue of amino acids for purposes other than protein synthesis has important effects on whole-body amino acid economy.

Sources of Amino Acids for Mucosal Protein Synthesis

The villous enterocytes are unique among body cells in that they are presented with substantial quantities of potential precursor amino acids from both the diet and the mesenteric arterial circulation. It has been known for many years that the cells of the mucosa are capable of utilizing both sources of amino acids for protein synthesis (1), but the source that is used under practical feeding conditions is not known with certainty. This is regrettable because the source of amino acids that is responsible for approximately 15% of total body protein turnover has both physiological and nutritional implications.

There is a substantial first-pass metabolism of dietary amino acids by the tissues of the splanchnic bed (12, 47, 69, 94). Using a combination of stable isotopically labeled amino acids and measurements of portal tracee and tracer uptake, Yu et al (110, 111) and Stoll et al (91, 92) have shown that in dogs and pigs, respectively, the intestine is responsible for >70% of this splanchnic first-pass metabolism. The results of both studies also show that there is continuing removal of arterial amino acids by the portal-drained viscera in the fed state. Furthermore, there appears to be channeling of arterial amino acids to mucosal constitutive protein synthesis. Working with piglets, Stoll et al (91) used simultaneous intragastric and intravenous infusions of an amino acid labeled with different stable isotopes and measured the relative isotopic enrichments of the two tracers in the mucosal free and protein-bound amino acids. Although both tracers were incorporated into the resident proteins of the mucosa, thereby confirming the results of Alpers (1), the results (Table 8) implied that arterial phenylalanine contributed 30% of the labeled mucosal free phenylalanine but 60% of the protein labeling was from the arterial tracer. In other words, there is a twofold greater chance of a phenylalanine molecule that enters the enterocyte

Table 8 The labeling of mucosal free and protein-bound phenylalanine in piglets fed a milk-replacer diet and infused intragastrically with [1-^{13}C]phenylalanine and intravenously with [*ring*-$^{2}H_5$]phenylalanine[a]

Determinant	Mucosal free phenylalanine	Mucosal protein-bound phenylalanine	Protein-bound: free
i.g. tracer	3.3 ± 0.3	1.1 ± 0.1	0.32 ± 0.04
i.v. tracer	0.48 ± 0.02	0.27 ± 0.02	0.56 ± 0.03
i.v./i.g.	0.15 ± 0.01	0.26 ± 0.03	

[a]i.g., Intragastric; i.v., intravenous. Results show mol percent excess. From Reference 91.

Table 9 Isotopic enrichment of leucine in plasma, mucosa, and prolactase in piglets fed with a conventional[a] or an elemental[b] diet and infused intravenously with [^{2}H]leucine[c]

Determinants	Plasma free leucine	Mucosal free leucine	Prolactase leucine
Conventional diet	4.0 ± 1.0	1.2 ± 0.2	1.8 ± 0.2
Elemental diet	4.9 ± 0.8	1.5 ± 0.9	2.2 ± 0.9

[a]H Wang, MA Dudley, PJ Reeds, unpublished data.
[b]From Reference 26.
[c]Results show mol percent excess.

via the basolateral membrane being incorporated into protein than one that enters the cell from the intestinal lumen. However, it is important to note that when account is taken of the relative molar uptake of amino acids from the diet and the mesenteric artery, dietary phenylalanine accounted for between 50% and 60% of total jejunal mucosal protein synthesis. There is also evidence for preferential incorporation of arterial leucine into prolactase (25, 26). This pro-polypeptide has an extremely high turnover rate and can be readily brought to isotopic equilibrium during an infusion of labeled amino acid. At plateau its labeling can be used to ascertain the relationship between the labeling of the free amino acid and protein synthetic precursor pools. Results from two recent experiments (26; Z Wang, MA Dudley, PJ Reeds, unpublished data) (Table 9) show that the plateau isotopic enrichment of prolactase is higher than that of the free amino acid pool, which suggests once again a preferential utilization of arterial leucine for mucosal protein synthesis.

The Catabolism of Amino Acids by Gastrointestinal Tissues

Enterocytes utilize glutamine to meet a significant proportion of their energy requirements (104, 105). This fact has generated a large literature on glutamine metabolism, but less than half the CO_2 production could be accounted for by glutamine alone and recent measurements of the portal balance of enterally infused [U-^{13}C]glutamate have shown that the metabolism of this amino acid may exceed that of glutamine by at least threefold (76). Other potential fuels are glucose, ketones, and amino acids in general, but the relative importance of these substrates and the changes that occur with feeding and fasting remain poorly established in vivo. The relative importance of these fuels may differ among species (88, 103).

Only a small fraction of glucose metabolized by enterocytes is fully oxidized (103). Most is converted to lactate or to pyruvate, which serves as precursor for the synthesis of alanine, an amino acid that is produced in large quantities by the mucosa (20, 76), itself a result that supports the idea of substantial catabolism of amino acids at this site. In experiments by Darcy-Vrillon et al (20) with growing

Table 10 Comparison of the amount of each amino acid consumed, the amount removed from the lumen of the small intestine, and the amount removed by the portal circulation in 50-kg pigs[a]

Amino acid	Intake	Uptake	Portal removal
Asx	12.9	12.0	22.3
Thr	7.2	6.6	8.3
Ser	9.4	8.5	13.8
Glx	39.5	37.9	3.5
Pro	19.7	19.1	21.4
Gly	3.4	2.8	10.3
Ala	5.4	4.9	26.8
Val	11.3	10.7	14.9
Ile	9.0	8.4	10.8
Leu	16.5	15.7	16.3
Tyr	6.6	6.0	10.7
Phe	9.5	9.2	9.8
Lys	13.3	12.9	20.1
His	4.9	4.8	6.1
Arg	6.3	6.0	10.2
Cys	0.9	0.7	1.3
Met	4.2	4.1	5.8

[a]Results show grams per 24 h. From Reference 20.

pigs, the amount of each amino acid leaving the small intestinal lumen was compared with that removed by the portal circulation. These studies showed that for all amino acids except glutamate + glutamine, portal appearance was substantially greater than prececal removal from the lumen. For the essential amino acids, this is consistent with the evidence from ^{15}N labeling that secreted proteins are recycled to the body. There was, however, a substantial variation among the dispensable amino acids (Table 10), reflecting the fact that whereas some are catabolized within the gut tissue, others are synthesized.

Results from a recent study using a combination of enteral infusions of [U-^{13}C]protein and measurements of portal amino acid, ammonia, and [U-^{13}C]tracer balance as well as protein incorporation (92) (Table 11) imply that the metabolism of dietary amino acids may be a major source of energy for the fed intestinal mucosa. In this study, between 40% and 70% of enteral tracer essential amino acids that had been removed from the lumen appeared in the portal blood. This contrasted with glucose appearance, which accounted for $>90\%$ of the intake. However, no more than 20% of the metabolized tracer amino acids was recovered in resident mucosal protein. Even if account is taken of protein secretion into the lumen, approximately 50% of the utilization of the dietary amino acids by the mucosa was directed to catabolism. This was confirmed by the observation that once feeding was instituted, there was a rapid and

Table 11 Estimates of first-pass utilization of selected dietary amino acids in young pigs[a]

Amino acid	Total first-pass utilization	Incorporation into mucosal protein[b]	Estimated mucosal catabolism
Glutamate	96 ± 4	10 ± 3	86 ± 7
Threonine	61 ± 15	26 ± 4	35 ± 11
Leucine	31 ± 5	12 ± 3	19 ± 4
Lysine	35 ± 12	12 ± 1	23 ± 6
Phenylalanine	35 ± 11	12 ± 2	23 ± 5

[a]Results show percentage utilization. Recalculated from Reference 76, Reference 92, and PJ Reeds, DG Burrin, B Stoll, F Jahoor, unpublished data.

[b]Assumption is that 50% of mucosal protein synthetic incorporation is secreted into the lumen.

substantial production of ammonia by the portal drained viscera that accounted for at least 20% of the dietary protein-nitrogen intake. Although these results await confirmation in other species, they imply that amino acid metabolism in general, and dietary amino acid metabolism in particular, may dominate the energy economy of the intestinal mucosa. This has substantial implications for the quantification of the bioavailability of dietary protein.

ACKNOWLEDGMENTS

This work was supported in part by the Scottish Office Agriculture, Environment and Fisheries Department. This publication is in part from the Agriculture/Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX. Funding has been provided in part from the US Department of Agriculture/Agricultural Research Service under Cooperative Agreement 5862-5-6-001. The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture. Mention of trade names, commercial products, or organizations does not imply endorsement by the US government.

Literature Cited

1. Alpers DH. 1972. Protein synthesis in intestinal mucosa: the effect of route of administration of precursor amino acids. *J. Clin. Invest.* 51:167–73
2. Aqvist SEG. 1952. Metabolic interrelationships between amino acids studied with isotopic nitrogen. *Acta Chem. Scand.* 5:1046–64
3. Aw TY, Williams MW. 1992. Intestinal absorption and lymphatic transport of peroxidized lipids in rats: effect of exogenous GSH. *Am. J. Physiol.* 263:G665–72

4. Baglieri A, Mahé S, Benomouzig R, Savoie L, Tomé D. 1995. Digestion patterns of endogenous and different exogenous proteins affect the composition of intestinal effluents in humans. *J. Nutr.* 125:1894–903
5. Baglieri A, Mahé S, Zidi S, Huneau J-F, Thullier F, et al. 1994. Gastro-jejunal digestion of soya-bean-milk in humans. *Br. J. Nutr.* 72:519–32
6. Bender DA. 1985. *Amino Acid Metabolism*. Chichester, NY: Wiley
7. Bergner H, Simon O, Zebrowska T, Münchmeyer R. 1986. Studies on the secretion of amino acids and urea into the gastrointestinal tract of pigs. 3. Secretion of urea determined by continuous intravenous infusion of ^{15}N-urea. *Arch. Tierernähr.* 36:479–490
8. Berthold HK, Hachey DL, Reeds PJ, Thomas OP, Hoeksema S, Klein PD. 1991. Uniformly ^{13}C-labeled algal protein used to determine amino acid essentiality in vivo. *Proc. Natl. Acad. Sci. USA* 88:8091–95
9. Berthold HK, Reeds PJ, Klein PD. 1995. Isotopic evidence for the differential regulation of arginine and proline synthesis in man. *Metabolism* 44:466–473
10. Bickerton AS, Birch R, Jackson AA, Uauy R, Persaud C, Gattas V. 1996. Protein quality and urea kinetics in prepubertal Chilean schoolboys. *Int. J. Food Sci. Nutr.* 47:61–70
11. Binder HJ. 1970. Amino acid absorption in the mammalian colon. *Biochim. Biophys. Acta* 219:503–6
12. Biolo G, Tessari P, Inchiostro S, Bruttomesso D, Frongher C. 1992. Leucine and phenylalanine kinetics during mixed meal ingestion: a multiple tracer approach. *Am. J. Physiol.* 262:E455–63
13. Boirie YM, Fauquant J, Rulquin H, Maubois JL, Beaufrere B. 1995. Production of large amounts of [^{13}C]leucine-enriched milk proteins by lactating cows. *J. Nutr.* 125:92–98
14. Butts CA, Moughan PJ, Smith WC. 1991. Endogenous amino acid flow at the terminal ileum of the rat determined under conditions of peptide alimentation. *J. Sci. Food Agric.* 55:175–87
15. Butts CA, Moughan PJ, Smith WC, Carr DH. 1993. Endogenous lysine and other amino acid flows at the terminal ileum of the growing pig (20 kg bodyweight): the effect of protein-free, synthetic amino acid, peptide and protein alimentation. *J. Sci. Food Agric.* 61:31–40
16. Coates ME, Fuller R. 1993. Microflora of the intestine. Role and effects. In *Encyclopaedia of Food Science, Food Technology and Nutrition*, ed. R Macrae, RK Robinson, MJ Sadler, pp. 3031–34. London: Academic
17. Corring T. 1980. Endogenous secretions in the pig. In *Current Concepts of Digestion and Absorption in Pigs*, ed. AG Low, IG Partridge, pp. 136–50. Reading, UK: Natl. Inst. Res. Dairy
18. Corring T, Calmes R, Rérat A, Geugneau A-M. 1984. Effects of short-term feeding of a protein-free diet on endogenous nitrogen secretion: exocrine pancreas secretion in the pig. *Reprod. Nutr. Dev.* 24:495–506
19. Corring T, Souffrant WB, Darcy-Vrillon B, Gebhardt G, Laplace JP, Rérat A. 1990. Exogenous and endogenous contribution to nitrogen fluxes in the digestive tract of pigs fed a casein diet. 1. Contribution of nitrogen from the exocrine pancreatic secretion and the bile. *Reprod. Nutr. Dev.* 30:717–722
20. Darcy-Vrillon B, Souffrant WB, Laplace JP, Rérat A, Corring T, et al. 1991. Exogenous and endogenous contributions to nitrogen fluxes in the digestive tract of pigs fed a casein diet. II. Ileal and faecal digestibilities and absorption of amino acids. *Reprod. Nutr. Dev.* 31:561–73
21. de Lange CFM, Sauer WC, Souffrant W. 1989. The effect of protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. *J. Anim. Sci.* 67:755–62
22. de Lange CFM, Sauer WC, Souffrant WB, Lien K. 1992. ^{15}N-leucine and ^{15}N-isoleucine isotope dilution techniques versus the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein and amino acids in digesta collected from the distal ileum in pigs. *J. Anim. Sci.* 70:1848–56
23. de Lange CFM, Souffrant WB, Sauer WC. 1990. Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N-isotope dilution technique. *J. Anim Sci.* 68:409–18
24. Drescher K, Hagemeister H, de Vrese M, Roos N, Middendorf K, Rantamäki P. 1994. Guadination (homoarginine-labelling) does not affect prececal ^{15}N recovery in pigs. In *Proc. 6th Int. Symp. Dig. Physiol. Pigs*, ed. WB Souffrant, H Hagemeister, pp. 64–66. Dummerstorf, Germany: Forsch.inst. Biol. Landwirtsch. Nutztiere
25. Dudley MA, Burrin DG, Quoroni A, Rosenberger J, Cook G, et al. 1996. Lactase Phlorhizin hydrolase turnover in vivo

in water-fed and colostrum-fed newborn pigs. *Biochem. J.* 320:735–43
26. Dudley MA, Wykes LJ, Dudley AW, Burrin DG, Nichols BL, et al. 1998. Parenteral nutrition selectively decreases protein synthesis in the small intestine. *Am. J. Physiol.* 274:G131–37
27. El-Khoury AE, Ajami AM, Fukagawa NK, Chapman TE, Young VR. 1996. Diurnal pattern of the interrelationships among leucine oxidation, urea production, and hydrolysis in humans. *Am. J. Physiol.* 271:E563–73
28. El-Khoury AE, Fukagawa NK, Sánchez M, Tsay RH, Gleason RE, et al. 1994. Validation of the tracer balance concept with reference to leucine: 24 hour intravenous tracer studies with L-(1-^{13}C)leucine and (^{15}N-^{15}N)urea. *Am. J. Clin. Nutr.* 59:1000–11
29. Fan MZ, Sauer WC. 1997. Determination of true ileal amino acid digestibility in feedstuffs for pigs with the linear relationships between distal ileal outputs and dietary inputs of amino acids. *J. Sci. Food Agric.* 73:189–99
30. Fan MZ, Sauer WC, Hardin RT, Lien KA. 1994. Determination of apparent ileal digestibility in pigs: effect of dietary amino acid level. *J. Anim. Sci.* 67:755–62
31. Forrester T, Badaloo AV, Persaud C, Jackson AA. 1994. Urea production and salvage during pregnancy in normal Jamaican women. *Am. J. Clin. Nutr.* 60: 341–46
32. Fuller MF, Garlick PJ. 1994. Human amino acid requirements: Can the controversy be resolved? *Annu. Rev. Nutr.* 14:217–41
33. Fuller MF, McWilliam R, Wang TC, Giles LR. 1989. The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. *Br. J. Nutr.* 62:255–67
34. Fuller MF, Milne A, Harris CI, Reid TMS, Keenan R. 1994. Amino acid losses in ileostomy fluid on a protein-free diet. *Am. J. Clin. Nutr.* 59:70–73
35. Fuller MF, Reeds PJ, Cadenhead A, Sève B, Preston T. 1987. Effects of the amount and quality of dietary protein on nitrogen metabolism and protein turnover of pigs. *Br. J. Nutr.* 58:287–300
36. Furst P. 1972. 15N studies in severe renal failure. 2. Evidence for the essentiality of histidine. *Scand. J. Clin. Lab. Invest.* 30: 307–12
37. Gargallo J, Zimmerman DR. 1981. Effect of casein and starch infusion in the large intestine on nitrogen metabolism of growing swine. *J. Nutr.* 111:1390–96
38. Gaudichon C, Laurent C, Mahé S, Marks L, Tomé D, et al. 1994. Rate of [^{15}N]leucine incorporation and determination of nitrogenous fractions from gastro-jejunal secretion in fasting humans. *Reprod. Nutr. Dev.* 34:349–59
39. Gaudichon C, Mahé S, Laurent C, Meaugeais P, Krempf M, et al. 1996. A ^{15}N-leucine-dilution method to measure endogenous distribution of luminal nitrogen in the human upper jejunum. *Eur. J. Clin. Nutr.* 50:261–68
40. Gaudichon C, Roos N, Mahé S, Sick H, Bouley C, et al. 1994. Gastric emptying regulates the kinetics of nitrogen absorption from ^{15}N-labeled milk and ^{15}N-labeled yogurt in miniature pigs. *J. Nutr.* 124:1970–77
41. Gausseres N, Mahé S, Benamouzig R, Luengo C, Drouet H, et al. 1996. The gastroileal digestion of ^{15}N-labelled pea nitrogen in adult humans. *Br. J. Nutr.* 76:75–85
42. Giordano C, de Pascale C, Balestrieri C, Cittadini D, Crescenzi A. 1968. The incorporation of urea ^{15}N in amino acids of patients with chronic renal failure on low nitrogen diet. *Am. J. Clin. Nutr.* 21:394–404
43. Gustafsson BE. 1984. The germ-free animal: its potential and its problems. In *The Germ-Free Animal in Biomedical Research*, ed. ME Coates, BE Gustafsson, pp. 1–8. London: Lab. Anim.
44. Hagemeister H, Erbersdobler HF. 1985. Chemical labelling of dietary protein by transformation of lysine to homoarginine: a new technique to follow intestinal digestion and absorption. *Proc. Nutr. Soc.* 44:133A (Abstr.)
45. Heine W, Wutzke KD, Richter I, Walther F, Plath C. 1987. Evidence for colonic absorption of protein nitrogen in infants. *Acta Paediatr. Scand.* 76:741–44
46. Herskovic T, Katz J, Floch MH, Spencer RP, Spiro HM. 1967. Small intestinal absorption and morphology in germ-free, monocontaminated and conventionalized mice. *Gastroenterology* 52:1136
47. Hoerr RA, Matthews DE, Bier DM, Young VR. 1991. Leucine kinetics from [$^{2}H_3$]- and [^{13}C]leucine infused simultaneously by gut and vein. *Am. J. Physiol.* 260:E111–17
48. Holt LE, Snyderman SE. 1972. The amino acid requirements of children. In *International Encyclopaedia of Food and Nutrition*. II. *Proteins and Amino Acid Functions*, ed. EJ Bigwood. Oxford, UK: Pergamon

49. Jackson AA. 1995. Salvage of urea-nitrogen and protein requirements. *Proc. Nutr. Soc.* 54:535–47
50. Jackson AA, Danielsen MS, Boyes S. 1993. A noninvasive method for measuring urea kinetics with a single dose of [^{15}N^{15}N]urea in free-living humans. *J. Nutr.* 123:2129–36
51. Jackson AA, Picou D, Landman JP. 1984. The non-invasive measurement of urea kinetics in normal man by a constant infusion of ^{15}N-^{15}N-urea. *Hum. Nutr. Clin. Nutr.* 38C:339–54
52. James PS, Smith MW. 1976. Methionine transport by pig colonic mucosa measured during early post-natal development. *J. Physiol.* 262:151–68
53. Jensen BB. 1988. Effect of diet composition and virginiamycin on microbial activity in the digestive tract of pigs. In *Digestive Physiology in the Pig*, ed. L Buraczewska, S Buraczewska, B Pastuzewska, T Zebrowska, pp. 392–400. Jabłonna, Poland: Polish Acad. Sci.
54. Juste C. 1982. Apports endogenes par les secretions digestives chez le porc. In *Physiol. Dig. Porc, 27–29 Oct*, ed. JP Laplace, T Corring, A Rérat, Jouy-en-Josas. Versailles, France: INRA
55. Kies CV, Fox HM. 1978. Urea as a dietary supplement for humans. In *Nutritional Improvement of Food and Feed Proteins. Advances in Experimental Medicine and Biology*, ed. M Friedman, 105:103–18. New York: Plenum
56. Kofrányi E, Jekat F. 1964. Determination of the biological value of food proteins. IX. Substitution of high-value proteins by non-essential nitrogen. *Z. Physiol. Chem.* 338:154–58
57. Krawielitzki K, Kreienbring F, Zebrowska T, Schadereit R, Kowalczyk J. 1994. Estimation of N absorption, secretion and reabsorption in different intestinal sections of growing pigs using the ^{15}N isotope dilution method. In *Proc. 6th Int. Symp. Dig. Physiol. Pigs*, ed. WB Souffrant, H Hagemeister, pp. 79–82. Dummerstorf, Germany: Forsch.inst. Biol. Landwirtsch. Nutztiere
58. Krawielitzki K, Zebrowska T, Schadereit R, Kowalczyk J, Hennig U, et al. 1990. Determining of nitrogen absorption and nitrogen secretion in different sections of the pig's intestine by digesta exchange between ^{15}N labelled and unlabelled animals. *Arch. Anim. Nutr.* 40:25–37
59. JP Laplace, Darcy-Vrillon B, Duval Y, Raibaud P. 1985. Proteins in the digesta of the pig-amino acid composition of endogenous, bacterial and fecal fractions. *Reprod. Nutr. Dev.* 25:1083–99
60. Leterme P, Monmart T, Morandi P, Thewis A. 1994. Effect of oral and parenteral nutrition vs N-free nutrition on the endogenous amino acid flow at the ileum of the pig. In *VI Int. Symp. Dig. Physiol. Pigs*, ed. W-B Souffrant, H Hagemeister, pp. 60–63. Dummersdorf/Rostock, Germany: Eur. Assoc. Anim. Prod.
61. Leterme P, Thewis A, Francois E, Van Leeuwen P, Wathelet B, Huisman J. 1996. The use of ^{15}N-labelled dietary proteins for determining true ileal amino acid digestibilities is limited by their rapid recycling in the endogenous secretions of pigs. *J. Nutr.* 126:2188–98
62. Lien KA, Sauer WC, Dugan MER. 1997. Evaluation of the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein in ileal digesta of pigs: effects of the pattern of blood sampling, precursor pools, and isotope dilution technique. *J. Anim. Sci.* 75:159–69
63. Lien KA, Sauer WC, Mosenthin R, Souffrant WB, Dugan MER. 1997. Evaluation of the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein in ileal digesta of pigs: effect of dilution in the precursor pool for endogenous nitrogen secretion. *J. Anim. Sci.* 75:148–58
64. Long CL, Jeevanandam M, Kinney TD. 1978. Metabolism and recycling of urea in man. *Am. J. Clin. Nutr.* 31:1367–82
65. Mahé S, Benamouzig R, Gaudichon C, Huneau J-P, De Cruz I, et al. 1995. Nitrogen movements in the upper jejunum in humans fed low amounts of casein and β-lactoglobumin. *Gastroenterol. Clin. Biol.* 19:20–26
66. Mahé S, Roos N, Benamouzig R, Davbin L, Luengo G, et al. 1996. Gastrojejunal kinetics and the digestion of [^{15}N]ß-lactoglobulin and casein in humans: the influence of the nature and quantity of the protein. *Am. J. Clin. Nutr.* 63:546–52
67. Mahé S, Roos N, Benamouzig R, Sick H, Baglieri A, et al. 1994. True exogenous and endogenous nitrogen fractions in the human jejunum after ingestion of small amounts of ^{15}N-labeled casein. *J. Nutr.* 124:548–55
68. Malmlöf K, Simoes Nunes C. 1992. The effects of intravenous urea infusions on portal and arterial plasma ammonia and urea enrichment of jejunal and colonic infusions. Acute experiments with growing pigs. *Scand. J. Gastroenterol.* 27:620–24
69. Matthews DE, Marano MA, Campbell

RG. 1993. Splanchnic bed utilization of leucine and phenylalanine in humans. *Am. J. Physiol.* 264:E109–18

70. Meakins TS, Jackson AA. 1996. Salvage of exogenous urea nitrogen enhances nitrogen balance in normal men consuming marginally inadequate protein diets. *Clin. Sci.* 90:215–25
71. Metges CC, Petzke KJ. 1997. Measurement of $^{15}N/^{14}N$ isotopic composition in individual plasma free amino acids of human adults at natural abundance by gas chromatography-combustion isotope ratio mass spectrometry. *Anal. Biochem.* 247:158–64
72. Metges CC, Petzke KJ, Bedri S, Fuller MF, Young VR. 1997. The quantitative contribution of microbial lysine to lysine flux in healthy male subjects. *FASEB J.* 11:A149
73. Mosenthin R, Sauer WC, de Lange CFM. 1992. Tracer studies of urea kinetics in growing pigs: I. The effect of intravenous infusion of urea on recycling and the site of urea secretion into the gastrointestinal tract. *J. Anim. Sci.* 70:3458–66
74. Olszewski A, Buraczewski S. 1978. Absorption of amino acids in isolated pig caecum in situ. Effect of concentration of enzymatic casein hydrolysate on absorption of amino acids. *Acta Physiol. Pol.* 29:67–77
75. Patterson BW, Carraro F, Klein SE, Wolfe RR. 1995. Quantification of incorporation of [^{15}N]ammonia into plasma amino acids and urea. *Am. J. Physiol.* 269:E508–15
76. Reeds PJ, Burrin DG, Jahoor PF, Wykes L, Henry J, Frazer EM. 1996. Enteral glutamate is almost completely metabolized in first pass by the gastrointestinal tract of infant pigs. *Am. J. Physiol.* 33:E413–18
77. Reeds PJ, Burrin DG, Stoll B, Jahoor F, Henry J, Frazer ME. 1997. Enteral glutamate is the preferential precursor for mucosal glutathione synthesis in the piglet. *Am. J. Physiol.* 273:E408–15
78. Reeds PJ, Fuller MF, Lobley GE, Cadenhead A, McDonald JD. 1978. Protein synthesis and amino acid oxidation in growing pigs. *Proc. Nutr. Soc.* 37:106A
79. Roos N, Mahé S, Benamouzig R, Sick H, Rautureau J, Tomé D. 1995. ^{15}N-labeled immunoglobulins from bovine colostrum are partially resistant to digestion in human intestine. *J. Nutr.* 125:1238–44
80. Sauer WC, Ozimek L. 1986. Digestibility of amino acids in swine: results and their practical application. A review. *Livestock Prod. Sci.* 15:367–88
81. Savage DC. 1977. Microbial ecology of the gastrointestinal tract. *Annu. Rev. Microbiol.* 31:107–133
82. Schoenheimer R, Ratner S. 1939. Studies in protein metabolism. 3. Synthesis of amino acids containing isotopic nitrogen. *J. Biol. Chem.* 127:301–13
83. Schulze H, Butts CA, Moughan PJ, Verstegen MWA. 1994. Endogenous ileal nitrogen flow in pigs determined using the ^{15}N-isotope dilution and peptide alimentation ultrafiltration methods. In *Proc. 6th Int. Symp. Dig. Physiol. Pigs*, ed. WB Souffrant, H Hagemeister, pp. 14–16. Dummerstorf, Germany: Forsch.inst. Biol. Landwirtsch. Nutztiere
84. Sepúlveda FV, Smith MW. 1979. Different mechanisms for neutral amino acid uptake by new-born pig colon. *J. Physiol.* 286:479–90
85. Sheng Y-B, Badger TM, Asplund JM, Wixom RL. 1977. Incorporation of $^{15}NH_4Cl$ into histidine in adult man. *J. Nutr.* 107:621–30
86. Shulman RJ, Gannon N, Reeds PJ. 1995. Cereal feeding and its impact on the nitrogen economy of the infant. *Am. J. Clin. Nutr.* 62:969–72
87. Smith MW, James PS. 1976. Amino acid transport by the helicoidal colon of the new-born pig. *Biochim. Biophys. Acta* 419:391–94
88. Souba WW, Scott TE, Wilmore DW. 1985. Intestinal consumption of intravenously administered fuels. *J. Parent. Ent. Nutr.* 9:18–22
89. Souffrant WB, Darcy-Vrillon B, Corring T, Laplace J-P, Koehler R, et al. 1986. Recycling of endogenous nitrogen in the pig. *Arch. Tierernähr.* 36:269–74
90. Souffrant WB, Rérat A, Laplace J-P, Darcy-Vrillon B, Kohler R, et al. 1993. Exogenous and endogenous contributions to nitrogen fluxes in the digestive tract of pigs fed a casein diet. III. Recycling of endogenous nitrogen. *Reprod. Nutr. Dev.* 33:373–82
91. Stoll B, Burrin DG, Henry J, Jahoor F, Reeds PJ. 1997. Phenylalanine utilization by the gut and liver measured with intravenous and intragastric tracers in pigs. *Am. J. Physiol.* 273:G1166–75
92. Stoll B, Henry J, Reeds PJ, Yu H, Burrin DG. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk-replacer fed piglets. *J. Nutr.* 128:606–14
93. Tanaka N, Kubo K, Shiraki K, Koishi H, Yoshimura H. 1980. A pilot study on protein metabolism in the Papua New

Guinea highlanders. *J. Nutr. Sci. Vitaminol.* 26:247–59
94. Tessari P, Garibotto G, Inchiostro R, Robaudo C, Saffioti S, et al. 1996. Kidney, splanchnic, and leg protein turnover in humans—insight from leucine and phenylalanine kinetics. *J. Clin. Invest.* 98:1481–92
95. Torrallardona D. 1994. *The contribution of the gastrointestinal microflora to the amino acid requirements of nonruminants*. PhD thesis. Univ. Aberdeen, Aberdeen, Scotland. 144 pp.
96. Torrallardona D, Harris CI, Coates ME, Fuller MF. 1996a. Microbial amino acid synthesis and utilization in rats: incorporation of ^{15}N from $^{15}NH_4Cl$ into lysine in the tissues of germ-free and conventional rats. *Br. J. Nutr.* 76:689–700
97. Torrallardona D, Harris CI, Fuller MF. 1996b. Microbial amino acid synthesis and utilization in rats: the role of coprophagy. *Br. J. Nutr.* 76:701–9
98. Torrallardona D, Harris CI, Milne E, Fuller MF. 1994. The contribution of intestinal microflora to amino acid requirements in pigs. In *Proc. 6th Int. Symp. Dig. Physiol. Pigs*, ed. WB Souffrant, H Hagemeister, pp. 245–48. Dummerstorf, Germany: Forsch.inst. Biol. Landwirtsch. Nutztiere
99. Torrallardona D, Harris CI, Milne E, Fuller MF. 1996. Site of absorption of lysine synthesis by the gastrointestinal microflora of pigs. In *Proc. VII Int. Symp. Protein Metab. Nutr.*, ed. AF Nunes, AV Portugal, JP Costa, JR Ribeiro, pp. 369–71. Santarém, Portugal: Eur. Assoc. Anim. Prod.
100. Van Bruchem J, Voigt J, Toos SC, Lammer-Weinhoven W, Schonhusen U, et al. 1997. Secretion and reabsorption of endogenous protein along the small intestine of sheep: estimates from ^{15}N-dilution of plasma non-protein-N. *Br. J. Nutr.* 77:273–86
101. Walser M, Bodenlos LJ. 1959. Urea metabolism in man. *J. Clin. Invest.* 38:1617–26
102. Waterlow JC, Garlick PJ, Millward DJ. 1978. *Protein Turnover in Mammalian Tissues and in the Whole Body*. Amsterdam: Elsevier/North-Holland Biomed.
103. Windmueller HG. 1982. Glutamine utilization by the small intestine. *Adv. Enzymol.* 53:202–37
104. Windmueller HG, Spaeth AE. 1978. Identification of ketone bodies and glutamine as the major respiratory fuels in vivo for postabsorptive rat small intestine. *J. Biol. Chem.* 253:69–76
105. Windmueller HG, Spaeth AE. 1980. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. Quantitative importance of glutamine, glutamate and aspartate. *J. Biol. Chem.* 255:107–12
106. Wrong OM, Vince AJ, Waterlow JC. 1985. The contribution of endogenous urea to faecal ammonia in man, determined by ^{15}N labelling of plasma urea. *Clin. Sci.* 68:193–99
107. Wu G. 1995. Urea synthesis in enterocytes of developing pigs. *Biochem. J.* 312:717–23
108. Wünsche J, Hennig U, Meinl M, Kreienbring F, Bock H-D. 1982. Investigations of the absorption and utilization of amino acids infused into the caecum of growing pigs. 1. N-balance measuring with regard to the utilization of lysine and isoleucine and the isoleucine requirement of growing pigs. *Arch. Tierernähr.* 32:337–48
109. Deleted in proof
110. Yu Y-M, Burke JF, Vogt JA, Chambers L, Young VR. 1992. Splanchnic and whole body L-[1–^{13}C,^{15}N]leucine kinetics in relation to enteral and parenteral amino acid supply. *Am. J. Physiol.* 262:E687–94
111. Yu Y-M, Wagner DA, Tredget EE, Walaszewski JA, Burke JF, Young VR. 1990. Quantitative role of splanchnic region in leucine metabolism: L-[1–^{13}C,^{15}N]leucine and substrate balance studies. *Am. J. Physiol.* 259:E36–51
112. Zebrowska T. 1973. Digestion and absorption of nitrogenous compounds in the large intestine of pigs. *Rocz. Nauk Rol.* 95B:85–90
113. Zebrowska T. 1975. The apparent digestibility of nitrogen and individual amino acids in the large intestine of pigs. *Rocz. Nauk Rol.* 97B:117–23
114. Zebrowska T, Low AG, Zebrowska H. 1983. Studies on gastric digestion of protein and carbohydrate, gastric secretion and exocrine pancreatic secretion in the growing pig. *Br. J. Nutr.* 49:401–10