Lysine synthesized by the gastrointestinal microflora of pigs is absorbed, mostly in the small intestine

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Torrallardona, David, C. Ian Harris, and Malcolm F. Fuller. Lysine synthesized by the gastrointestinal microflora of pigs is absorbed, mostly in the small intestine. Am J Physiol Endocrinol Metab 284: E1177–E1180, 2003. First published February 4, 2003; 10.1152/ajpendo.00465.2002.—This study used a digesta transfer protocol to determine the site of absorption of lysine synthesized by the gastrointestinal microflora of pigs. Eight pigs were used, four with reentrant cannulas in the terminal ileum, two with simple T cannulas in the terminal ileum, and two intact. All pigs were given, for 5 days, the same low-protein diet that included fermentable carbohydrates. The diet of two pigs with reentrant cannulas (donor) and of the two intact (control) pigs was supplemented with 15NH4Cl. The two other pigs with reentrant cannulas (acceptor pigs) and those with simple T cannulas (used to supply unlabeled digesta) were given the same diet but unlabeled NH4Cl. Ileal digesta were collected continuously from all of the reentrant cannulas and kept on ice. All digesta from each donor pig were reheated and returned to the distal cannula of its companion acceptor, whose ileal digesta were discarded. Unlabeled ileal digesta from the pigs with simple cannulas were instilled into the distal cannula of the donor pigs. At the end of the experiment, the average 15N enrichment in the plasma free lysine of control pigs was 0.0407 atom % excess (APE); that of acceptor pigs was 0.0322 APE (79% of controls), whereas that of donor pigs was 0.0096 APE (24% of controls). Due to nitrogen recycling, acceptor pigs had labeled lysine in the digesta of the stomach only 0.0096 APE (24% of controls). Because mammals have no mechanism for the transamination of lysine, the labeling of body lysine from dietary [15N]ammonium chloride has been taken as evidence for the absorption of essential amino acids synthesized by the gastrointestinal microflora in both pigs (13) and human subjects (4). These findings were extended by showing the absorption in pigs of essential amino acids labeled with 14C from oral [14C]polyglucose (13). However, the calculation of the amounts of microbial amino acids absorbed depends crucially on the labeling of microbial amino acids at the site of absorption, and because the isotopic enrichment of microbial amino acids is much greater in the large intestine than in the small intestine, it is important to establish where microbially synthesized amino acids are absorbed. Studies in intact animals cannot resolve this question, and we have instead transferred labeled and unlabeled digesta between the small and large intestines of pigs and observed the consequent labeling of lysine in body protein.

MATERIALS AND METHODS

Principle of the experiment. The principle of the experiment is illustrated in Fig. 1. Two pigs, each with a reentrant cannula in the terminal ileum, acted as donor and acceptor, and an intact pig acted as the control. The donor and the control pig were given a diet with [15N]ammonium chloride; the acceptor pigs got the same diet but unlabeled. All of the digesta leaving the proximal cannula of the donor pig were collected and infused into the distal cannula of the acceptor pig, whose ileal digesta were discarded. To replace the digesta of the donor pig, an equal volume of unlabeled ileal digesta from a fourth pig was instilled into the distal cannula of the donor. This design was duplicated, with a total use of eight pigs.

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The experiments were conducted after approval by the Ethical Committee for Animal Experimentation of the Rowett Research Institute.

**Animals and surgery.** Eight female Cotswold crossbred pigs of 25.0 ± 0.26 (SE) kg body wt were chosen from the Rowett Research Institute’s herd. Six of these underwent surgery, under halothane anesthesia, for the placement of cannulas in the terminal ileum. In two of the pigs (donors of unlabeled digesta), a simple T cannula was placed 15 cm from the ileocecal junction. In another four pigs (donors and acceptors of labeled digesta), the ileum was transected 15 cm from the ileocecal junction. The cut ends were ligated with purse-string sutures, and a single polyvinyl chloride cannula was placed in each end. All of the cannulas were exteriorized through the flank. The remaining two pigs were left intact. After surgery, the animals were allowed to recover for 9 days, during which the experimental diet (unlabeled) was offered in gradually increasing quantities. By the 7th day after surgery, these animals were eating as much as their intact partners. The two pigs fitted with a simple T cannula were housed in individual pens; they were simply used as a source of unlabeled ileal digesta. All of the other pigs were kept in metabolism crates to facilitate digesta transfer and to deny them access to feces or to digesta from the cannula.

**Diet and feeding.** A low-protein diet containing fermentable carbohydrate (raw potato starch) was used (Table 1). During the 5-day labeling period, the diet was supplemented with $^{15}\text{NH}_4\text{Cl}$ (99 atoms %; total dose 5 g/pig) or with unlabeled NH$_4\text{Cl}$. All of the pigs received their feed in two daily meals, 600 g at 0800 and 300 g at 1400.

**Experimental procedures.** During the 9 days between surgery and the start of the experiment, all of the animals were given the unlabeled diet. All digesta flowed from the proximal cannula were collected continuously (24 h/day) on ice. At regular intervals, the digesta were reheated to 37°C in a water bath and reintroduced into the distal cannula of the same pig. During the 5-day labeling period, the ileal digesta of pigs were exchanged as shown in Fig. 1. Aliquots of ileal digesta from donor and acceptor pigs (corresponding to 5% of weight) were taken, pooled for each day, and kept frozen until required for analysis. The feces of the six pigs were collected quantitatively each day and kept frozen until required for analysis.

On the 6th day after the start of labeling, the animals were killed under halothane anesthesia, and their blood was collected in heparin (15,000 IU/l). The digestive contents of the stomach, the whole small intestine, and the cecum were sampled and frozen.

**Sample processing and lysine isolation and analysis.** The microbial fractions of feces and digesta were obtained by filtration and differential centrifugation, as described by Torrallardona et al. (12). The pellet containing the microbial fraction was hydrolyzed under reflux with 6 M HCl at 137°C for 18 h.

The $^{15}\text{N}$ enrichment in the lysine of the microbial hydrolysates was measured by gas chromatography-combustion-isotope ratio mass spectrometry. Briefly, the hydrolyzed amino acid sample (as the tert-butyldimethylsilyl derivative) was injected in splitless mode onto an HP 5890 gas chromatograph interfaced with a combustion module (Orchid; Europa Scientific, Crewe, UK). The individual amino acids were separated in the gas chromatograph and oxidized as they passed through a quartz tube containing platinumized copper wire (600°C). Carbon dioxide was removed by a liquid nitrogen trap, and the resulting N gas was admitted to a SIRA Series II gas isotope ratio mass spectrometer (VG Isotech, Middlewich, Cheshire, UK). The $^{15}\text{N}$ enrichment in the amino acid was determined by measuring the mass-to-charge ratio (m/z) 29/28 and comparing this to the same ratio in a gas of known isotopic content.

The plasma fraction of the blood was obtained by centrifugation at 2,000 g for 20 min. Plasma protein was precipitated with the addition of 1 M perchloric acid (PCA; 1:1 vol/vol) followed by centrifugation at 5,000 g for 30 min. PCA in excess was precipitated with the addition of 2 M KOH (up to pH 5.5) at 0°C. The supernatant was concentrated by rotoevaporation, and plasma free lysine was separated by preparative ion exchange (11). After desalting, lysine was digested to ammonium sulfate by a micro-Kjeldahl method, the ammonia was distilled, and the $^{15}\text{N}$ enrichments were measured by gas isotope ratio mass spectrometry (SIRA 12; VG Isogas) as described by Torrallardona et al. (12).

**Statistical analysis.** The $^{15}\text{N}$ enrichments in lysine of plasma and microbial protein of control, donor, and acceptor pigs were compared by ANOVA using the General Linear Model procedure of the Statistical Analysis System Institute (9).

**Table 1. Composition of diet as fed**

<table>
<thead>
<tr>
<th>Component</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch</td>
<td>355</td>
</tr>
<tr>
<td>Raw potato starch</td>
<td>300</td>
</tr>
<tr>
<td>Cellulose</td>
<td>100</td>
</tr>
<tr>
<td>Sugar</td>
<td>100</td>
</tr>
<tr>
<td>Casein</td>
<td>50</td>
</tr>
<tr>
<td>Soya oil</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin-mineral Premix</td>
<td>57</td>
</tr>
</tbody>
</table>

*Premix contained (per kg diet) 10,000 IU vitamin A, 2,000 IU vitamin D, 15 IU vitamin E, 12 mg vitamin C, 1.5 mg vitamin K, 5.4 mg vitamin B$_2$, 5.7 mg vitamin B$_6$, 0.038 mg vitamin B$_12$, 16.8 mg pantothenic acid, 29.1 mg nicotinic acid, 2.7 mg folie acid, 1,150 mg choline, 2.5 mg biotin, 2 mg thiamin, 150 mg inositol, 117 mg Fe, 100 mg Zn, 40 mg Mn, 175 mg Cu, 2 mg I, 1 mg Co, 0.25 mg Se, 420 mg Mg, 3.9 g K, 2.1 g Cl, 1.4 g Na, 3.4 g P, and 4.8 g Ca.
RESULTS

Labeling of plasma free lysine. The $^{15}$N enrichment of plasma free lysine (Table 2) was, on average, three times higher in the donor pigs than in the acceptors ($P < 0.05$). The sum of the enrichments in donors and acceptors (0.0418 atoms % excess (APE)) was similar to that of plasma lysine from the intact control animals (0.0407 APE).

Labeling of microbial lysine. The $^{15}$N enrichment of microbial lysine in the ileal digesta of the donor and acceptor pigs is shown in Fig. 2. Labeling in the pigs that received labeled diet (donors) increased between days 1 and 3 and then reached a plateau. In the acceptor pigs receiving unlabeled diet, there was hardly any increase in enrichment between days 1 and 5. On days 4 and 5, the $^{15}$N enrichment of microbial lysine in the ileal digesta of the donors was 18-fold higher ($P < 0.01$) than that of the acceptors.

The $^{15}$N enrichment of microbial lysine in feces is shown in Fig. 3. It was highest in the control pigs, followed by the acceptor pigs and donor pigs. There were significant differences ($P < 0.05$) between the groups as early as day 1, and these differences increased as the experiment progressed.

The $^{15}$N enrichment of microbial lysine in digesta from the stomach, small intestine, and large intestine at the end of the experiment is shown in Table 3. In the control pigs, as observed previously (13), microbial lysine enrichment was 12-fold higher in the large intestine than in the small intestine. Microbial lysine enrichment in the stomach was highly variable, and...
there were no significant differences among the groups (P > 0.05). The enrichment of microbial lysine in the small intestine of acceptor pigs was very low. In cecal digesta, the microbial lysine of control pigs was significantly more enriched than in either acceptors or donors (P < 0.05).

**DISCUSSION**

This experiment was designed to separate the roles of the upper and lower digestive tract in the absorption of microbially synthesized amino acids. The 15N labeling of plasma lysine in the donors was 0.032 and in acceptors 0.010, and the sum of the two was close to that of controls (0.041), suggesting that three-quarters of the absorption of label were in the small intestine and one quarter was in the large intestine.

The physical transection of the two parts of the gut cannot, however, isolate them metabolically, because nitrogenous substances absorbed in one part can be secreted into the other. For example, acceptor pigs, which received 15N only via digesta instilled into the cecum, nevertheless had labeled microbial lysine in the stomach and small intestine. Likewise, the donor pigs, which had unlabelled digesta instilled into the large intestine, nevertheless had highly labeled cecal digesta. Thus some of the labeled lysine in the plasma of donors was absorbed in the large intestine, and some of that in acceptors was absorbed in the small intestine.

The absorption of [15N]lysine (A) can be expressed as

\[ A = (S \times E_s) + (L \times E_l) \]

where S and L are the proportions absorbed in the small and large intestine, and \( E_s \) and \( E_l \) are the corresponding enrichments of the microbial lysine absorbed.

Since \( S + L = 1 \), and A for donors was 3.3 times that for acceptors, then

\[ (0.039 \times S) + (0.183 \times (1 - S)) = 3.3 \]

\[ \times (0.006 \times S) + (0.412 \times (1 - S)) \]

which gives \( S = 0.98 \).

The enrichments used for the small intestine are those measured in the microbial fraction of digesta taken from the whole small intestine. For acceptors, this is similar to the value measured in ileal outflow on day 5, but in donors, microbial lysine in ileal outflow had an enrichment of 0.11 APE, much higher than that in the pooled contents of the whole small intestine. We do not know exactly the enrichment of microbial lysine absorbed from the small intestine. If a value of 0.11 is used in the calculation above, it is estimated that 93% of microbial lysine was absorbed in the small intestine. The true value probably lies between the values of 0.93 and 0.98, but in either case, it seems that, despite its larger microbial population and higher isotopic enrichment, the large intestine has a very small role in the absorption of microbial lysine.

This accords with previous results in pigs in which microbial lysine was doubly labeled by giving [14C]polyglucose and [15N]ammonium chloride. The ratio of these isotopes in body lysine corresponded much more closely with the ratio in microbial lysine from the ileum than with that from the large intestine (13).

We conclude that amino acids synthesized by the gastrointestinal microflora of pigs are absorbed from the gut predominantly in the small intestine.

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