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Rate at which glutamine enters TCA cycle influences carbon atom fate in intestinal epithelial cells

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Quan, J., M. D. Fitch, and S. E. Fleming. Rate at which glutamine enters TCA cycle influences carbon atom fate in intestinal epithelial cells. *Am. J. Physiol.* 275 (*Gastrointest. Liver Physiol.* 38): G1299–G1308, 1998.—Glutamine carbon entry into the tricarboxylic acid (TCA) cycle was assessed in small intestinal epithelial cells by measuring CO₂ production from [1-¹⁴C]glutamine, and these data together with [U-¹⁴C]glutamine data were used to calculate fractional oxidation rates for glutamine. CO₂ production from either [1-¹⁴C]glutamine or [U-¹⁴C]glutamine showed saturation kinetics, and the concentration needed to achieve the half-maximal rate of CO₂ production was 0.7 and 0.4 mmol/l, respectively. Maximal rate for [1-¹⁴C]glutamine was twice that for [U-¹⁴C]glutamine. Increasing glutamine concentration did not cause proportional increases in glutamine entry into the TCA cycle and glutamine oxidation. Consequently, fractional oxidation of glutamine decreased with increasing glutamine concentration. Fractional oxidation could be predicted from the rate at which glutamine carbon entered the TCA cycle. (Aminoxy)-acetic acid, an aminotransferase inhibitor, reduced entry of glutamine into the TCA cycle and increased fractional oxidation of glutamine. Glutamate carbon entered the TCA cycle at about one-half the rate of glutamine-derived glutamate carbon and had a higher fractional oxidation rate when provided at equivalent concentrations to glutamine. These differences in the rate of entry predictably account for the differences in the metabolic fate of glutamine vs. glutamate carbon.

jejunum; anaplerosis; energy; alanine; enterocyte

THE CLINICAL IMPORTANCE OF glutamine to intestinal health has been extensively studied over the last decade. This interest was stimulated by earlier observations that glutamine was an important energy-producing substrate for intestinal epithelial cells. Studies (28, 29, 30, 31) performed in vivo demonstrated that glutamine is an essential respiratory substrate for cells in the small intestinal mucosa, accounting for over one-third of the total CO₂ produced in the small intestine. In vitro experiments have shown that jejunal epithelial cells produce more CO₂ from glutamine than from glucose (8, 9) and, when present together, glutamine and glucose produce similar amounts of ATP (11).

Despite the established importance of glutamine as an energy-providing substrate, reports of the effects of glutamine on intestinal structure and function have been inconsistent. Intestinal structure and function were found to be maintained or improved by glutamine supplementation in some studies using laboratory animals (5, 22, 33), but not in other studies (21, 32, 34). Also, glutamine supplementation has been concluded to be beneficial to the intestinal health of humans in some review articles (35) but not in others (4). Explanations for the inconsistent results have not been readily available.

Although glutamine provides energy for the intestinal mucosa, previous work (10, 15, 18) using the CO₂ ratios technique has suggested that glutamine is not completely oxidized to CO₂. As a consequence, glutamine carbon must efflux from the tricarboxylic acid (TCA) cycle and be incorporated into synthetic products. In support of this, glutamine carbon has been shown to be metabolized in vivo to CO₂, amino acids, and organic acids, including citrate and lactate (28, 31). Through the use of isolated cells, glutamine carbon has been found in metabolites, including glutamate, CO₂, lactate, alanine, aspartate, citrulline, proline, succinate, and ornithine (11, 26). The most likely pathway by which glutamine carbon could be incorporated into several of these metabolites would be via efflux of intermediates from the TCA cycle. Other minor but physiologically important synthetic products, such as lipids, would not have been quantified with the methodologies employed in these studies, although glutamine would be expected to provide precursors for the synthesis of any compounds derived from TCA cycle intermediates.

The major objective of these studies was to further evaluate whether or not the metabolic fate of glutamine carbon is influenced by changing the rate at which glutamine carbon enters the TCA cycle, as previous studies have suggested (15). These earlier studies (10, 15) were based on values for "A + T" (where A is the probability that carbon entering the TCA cycle will be incorporated into citrate via acetyl-CoA and T is the probability that carbon will be incorporated into citrate via oxaloacetate) derived using the CO₂ ratios approach. In the current study, our first approach was to measure CO₂ production from [1-¹⁴C]glutamine and [U-¹⁴C]glutamine to quantify glutamine carbon entry into the TCA cycle and glutamine oxidation, respectively. These data were used to calculate the fraction of carbon atoms entering the TCA cycle that are metabolized to CO₂, termed "fractional oxidation" of glutamine. A second approach was to quantify incorporation of [¹⁴C]glutamine into compounds produced via the TCA cycle. The results suggest that the metabolic fate of glutamine carbon is a function of the rate at which glutamine carbon enters the TCA cycle in isolated intestinal epithelial cells.

MATERIALS AND METHODS

Animals. Male Fischer 344 rats (Simonsen Laboratories, Gilroy, CA, or National Institute on Aging breeding colony, Harlan Industries, Indianapolis, IN) weighing 265–315 g were allowed access to commercial diets (Rat Chow no. 5012, Ralston Purina, St. Louis, MO, or NIH 31 stock diet, Western Research Products, Hayward, CA). All animals were allowed free access to diet and water. Animal handling procedures

were approved by the Animal Care and Use Committee at the University of California (Berkeley, CA).

Chemicals. Radiochemicals, purchased from DuPont NEN (Boston, MA) included L-[U-¹⁴C]glutamate, L-[1-¹⁴C]glutamate, L-[U-¹⁴C]glutamine, [1,4-¹⁴C]succinate, and [2,3-¹⁴C]succinate. Tracers were purified using conventional TLC (MN 300 Cellulose, Brinkman Instruments, Westbury, NY) and developed in a mixture of butanol, acetic acid, and water (24:4:10). Labeled substances were detected using a radioactive plate scanner (Bioscan, Washington, DC). The appropriate region of the plate was scraped to separate the tracer from the impurities. The cellulose containing the purified tracer was stored at -20°C, then eluted with Krebs-Henseleit (KH) buffer on the day of experimentation. The Ca²⁺-free KH buffer contained the following (in mmol/l): 121.3 NaCl, 4.85 KCl, 1.21 KH₂PO₄, 1.21 MgSO₄, and 25.5 NaHCO₃.

Reagent grade chemicals were obtained from Sigma Chemical (St. Louis, MO). These included antibiotics, antimycotics (streptomycin sulfate, penicillin G, kanamycin monosulfate, and amphotericin B), glutamine synthetase, lactate dehydrogenase assay kits, (aminoxy)acetic acid (AOA), and 2-amino-2-norbornane-carboxylic acid (BCH; also known as 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid). BSA (fraction V, pH 7.0, lyophilized) was purchased from ICN Biochemicals, and dinitrophenol was purchased from Aldrich (Milwaukee, WI).

Synthesis of L-[1-¹⁴C]glutamine. L-[1-¹⁴C]glutamine was synthesized according to the procedure of Brosnan and Hall (3). L-[1-¹⁴C]glutamate (45 μCi/μmol; 10 μCi) was added to 7 ml of an incubation medium (pH 7.1) containing 50 mmol/l imidazole hydrochloride, 20 mmol/l MgCl₂, 100 mmol/l NH₄Cl, 25 mmol/l mercaptoethanol, 10 mmol/l ATP, and 50 U glutamine synthetase and incubated at 37°C for 2 h. The entire incubation mixture was passed through a Dowex 1 Formate column, and the glutamine was eluted with 3 ml of water and stored at -20°C until used. Purity of the labeled glutamine was confirmed by TLC. Recovery of L-[1-¹⁴C]glutamine ranged from 92 to 96%.

Preparation of isolated cells. On the day of experimentation, animals were anesthetized by intraperitoneal injection of Nembutal (Abbott Laboratories, North Chicago, IL) at 5 mg/100 g rat, and the intestines were accessed via a midline incision. Cells from the proximal small intestine were prepared from a 30-cm segment beginning 10 cm distal to the pylorus; cells from the distal small intestine were prepared from the 25-cm segment proximal to the ileocecal junction. These segments were removed, and the animals were killed by exsanguination and thoracotomy.

The lumen of the isolated segment was washed free of contents. Then the cells were isolated from the mucosa, using a chemical and mechanical technique (9, 23), and modified to include antibiotics and antimycotics (11). The everted segments were filled with a Ca²⁺-free KH buffer containing 0.025% BSA, tied off, and incubated in 5 mmol/l EDTA at 37°C for 10 min for the proximal segment and 20 min for the distal segment. The cells were removed with a pressurized stream of ice-cold cell suspension buffer (KH buffer supplemented with CaCl₂ to 3.55 mmol/l, 0.25% BSA, 5 mmol/l dithiothreitol, and antibiotics as described above). The cell suspensions were centrifuged at low speed to sediment intact villi and crypts and to remove free cells in the supernatant. The final cell suspensions were kept on ice until the CO₂ assays began. Dry weights of cells were determined by heating at 100°C for 2 h and were calculated as the difference between the weight of 1 ml of cell suspension and 1 ml of Ca²⁺-containing KH buffer. Dry weights of cells ranged from 0.3–4.0 mg/flask.

Lactate dehydrogenase release (Sigma Diagnostics kit DG 1340-UV) was used to evaluate membrane integrity, as de-

scribed in detail previously (10). During the 25- to 30-min incubations, leakage of LDH into the medium averaged 5–11% of the total cell content.

CO₂ production. CO₂ production was measured as described previously (8). Aliquots of cell suspension were added to 25-ml Erlenmeyer flasks containing substrate (unlabeled substrate and tracer, in cell suspension buffer) and gassed with 95% O₂-5% CO₂. For most treatments, flasks contained 2–4 mg dry wt cells in 2 ml substrate medium. When treatments required using substrate at concentrations of 1 mmol/l or less, changes in substrate concentration due to its metabolism could be avoided by reducing cell weights to 0.3 mg/flask, increasing medium volume to 4 ml, and restricting incubation time to 25 min. Flasks were sealed with stoppers containing plastic center wells and incubated at 37°C for 25–30 min. After the incubation, the reactions were stopped by injecting 0.8 ml methanol and 1 ml NaH₂PO₄ (1 mol/l) into the flasks. CO₂ evolved during the incubation was trapped by adding 0.45 ml 10 mol/l NaOH to the center wells. After 2 h, the center wells were transferred to plastic scintillation vials containing 1 ml water and 15 ml scintillation fluid (Hionic Fluor, Packard Instruments, Downers Grove, IL) and placed in a scintillation counter for radioactivity measurements.

Total CO₂ production (in μmol CO₂ produced · g⁻¹ · min⁻¹) from [U-¹⁴C]glutamine was calculated as follows

$$\text{total CO}_2 \text{ production} = \left[\frac{\text{dpm } ^{14}\text{CO}_2/\text{ml cell suspension}}{n/(t \times C \times S)} \right]$$

where *t* is the incubation time in minutes, *C* is the dry weight of the cells (in g dry wt/ml cell suspension), *S* is the specific activity of the substrate (in dpm/mol substrate), and *n* is the number of carbon atoms per molecule of substrate.

A parallel equation was used to calculate the entry of glutamine carbon atoms (given in μmol carbon atoms · g⁻¹ · min⁻¹) into the TCA cycle. In this case, however, glutamine was labeled only in the carbon-1 position.

Values for fractional oxidation were calculated using the following equation

$$\text{fractional oxidation} = \frac{(\mu\text{mol CO}_2 \text{ produced} \cdot \text{g}^{-1} \cdot \text{min}^{-1})}{(\text{entry of } \mu\text{mol carbon atoms} \cdot \text{g}^{-1} \cdot \text{min}^{-1})}$$

where the numerator represents the oxidation of glutamine (using [U-¹⁴C]glutamine or [U-¹⁴C]glutamate) and the denominator equals entry of glutamine into the TCA cycle (using [1-¹⁴C]glutamine or [1-¹⁴C]glutamate). With [U-¹⁴C]glutamine as the tracer, specific activities ranged from 3 × 10⁵ dpm/μmol for glutamine at 5 mmol/l to 3 × 10⁶ dpm/μmol for glutamine at 0.1 mmol/l. With [1-¹⁴C]glutamine as the tracer, specific activities ranged from 1 × 10⁵ dpm/μmol for glutamine at 5 mmol/l to 5 × 10⁴ dpm/μmol for glutamine at 0.1 mmol/l.

Values for A + T were calculated using the formula derived by Kelleher (13) and Mallet et al. (18) as follows

$$A + T = \frac{(2 \times ^{14}\text{CO}_2 \text{ from } [2,3\text{-}^{14}\text{C}]\text{succinate})}{(^{14}\text{CO}_2 \text{ from } [1,4\text{-}^{14}\text{C}]\text{succinate} + ^{14}\text{CO}_2 \text{ from } [2,3\text{-}^{14}\text{C}]\text{succinate})}$$

Care was taken to ensure that equivalent quantities of these two tracers were added to flasks. Tracer was added at ~1 × 10⁷ dpm/flask.

Incorporation of glutamine carbon into metabolites. Epithelial cells were incubated with substrates and trace quantities of [U-¹⁴C]glutamine for 30 min as described above, then metabolism was stopped with 3 × vol 100% methanol. Each

incubation included 2–4 mg cells (dry wt), tracer (6×10^6 dpm), and 5 mmol/l glutamine or 5 mmol/l glutamine plus 2 mmol/l AOA. The production of CO_2 was determined in one set of flasks using the procedures described above. Incorporation of glutamine carbon into non- CO_2 metabolites was determined in a second set of flasks.

To determine the incorporation of substrate into non- CO_2 metabolites, we centrifuged the alcoholic incubates at 4,300 g for 2 min. Supernatants were lyophilized and extracted three times with 93% ethanol. The ethanol extracts were applied to 20×20 cm cellulose TLC plates and chromatographed using two-dimensional chromatography (*solvent 1* composed of *n*-butanol, acetic acid, and H_2O , 24:8:6, vol/vol/vol; *solvent 2* composed of phenol, H_2O , and NaCN, 3:1:0.003, wt/vol/wt; Ref. 28). The R_f values for key amino and key organic acids were previously determined. Samples were spiked with a mixture of unlabeled amino acids to facilitate visualization by spraying the developed plates with 0.05% fluorescamine (28). Radioactivity was visualized using a radioactive plate scanner. Regions of the plate corresponding to known metabolites were scraped and placed in scintillation vials, and compounds were eluted with 500 μl of 0.1 mmol/l NaOH, 1 ml H_2O , and aqueous compatible scintillation cocktail (Hionic Fluor). The specific activity of substrate (dpm/ μmol carbon of substrate = dpm $\cdot \mu\text{mol}$ substrate $^{-1}$ \cdot no. of carbons per molecule of substrate $^{-1}$) and the amount of radioactivity in each metabolite were used to calculate the incorporation of substrate carbon into metabolites.

Experimental design and statistical analysis. Figure 1 depicts the pathways involved and the metabolites examined in the eight experiments performed in this study.

The objective of *experiment 1* was to determine whether fractional oxidation of glutamine could be reliably measured using $[\text{U-}^{14}\text{C}]$ glutamine to measure oxidation to CO_2 and using $[\text{1-}^{14}\text{C}]$ glutamate to measure glutamine carbon entry into the TCA cycle. In this same experiment, cells were incubated with $[\text{1,4-}^{14}\text{C}]$ succinate and $[\text{2,3-}^{14}\text{C}]$ succinate to simultaneously determine values for A + T, facilitating comparisons between the two approaches. In this experiment, 14 rats were used and observations per treatment ranged from three to eight.

The objective of *experiments 2* and *3* was to evaluate the kinetics of glutamine entry into the TCA cycle and its oxidation to CO_2 . To do this, we incubated cells with either $[\text{1-}^{14}\text{C}]$ glutamine or $[\text{U-}^{14}\text{C}]$ glutamine as tracers. Glutamine concentrations ranged from 0.1 to 20 mmol/l in *experiment 2* and from 0.01 to 5 mmol/l in *experiment 3*. In both experiments, cells from three animals were exposed to all treat-

ments. The Lineweaver-Burk equation was used to calculate values for maximal rate (V_{max}) and the glutamine concentration needed to achieve the half-maximal rate of CO_2 production from either $[\text{U-}^{14}\text{C}]$ glutamine or $[\text{1-}^{14}\text{C}]$ glutamine (K_{ox}).

The objective of *experiment 4* was to determine whether reducing entry of glutamine carbon into the TCA cycle with a transaminase inhibitor, AOA, would increase the fractional oxidation of glutamine as predicted from the relationship between these variables that was determined in the previous experiments. AOA is known to be a general inhibitor of aminotransferases and other pyridoxal phosphate-dependent enzymes (12), but alanine aminotransferase and aspartate aminotransferase are the only pyridoxal phosphate-dependent enzymes known to be important for the metabolism of glutamine. AOA has been used previously in studies of enterocyte metabolism (2, 16, 19) and has been shown to reduce the relative rate of glutamine carbon entry into the TCA cycle using the CO_2 ratios approach (15). In this experiment, cells were incubated with either $[\text{1-}^{14}\text{C}]$ glutamine or $[\text{U-}^{14}\text{C}]$ glutamine as tracers, with either 5 mmol/l glutamine or 5 mmol/l each of glutamine and glucose, and in either the absence or presence of 2 mmol/l AOA. Cell suspensions were prepared from four animals, and each cell suspension was exposed to the eight treatments.

Experiment 5 was designed to expand on the objective of *experiment 4*. In *experiment 5*, however, incorporation of glutamine carbon into CO_2 as well as into non- CO_2 metabolites was quantified since this provided an alternative approach to assessing changes in fractional oxidation. To do this, we incubated cells, with $[\text{U-}^{14}\text{C}]$ glutamine and 5 mmol/l glutamine in either the absence or presence of 2 mmol/l AOA. Cell suspensions were prepared from four animals, and each cell suspension was exposed to the eight treatments.

The objective of *experiment 6* was to determine whether stimulating the entry of glutamate carbon into the TCA cycle would decrease fractional oxidation of glutamine, as was predicted by the hypothesis developed from the previous data. Because no stimulators of aminotransferase activity were known, we approached this with the glutamate dehydrogenase (GDH) stimulator BCH. Although glutamine carbon has been thought to enter the TCA cycle primarily via aminotransferase pathways, there is evidence of flux through GDH (2, 6). Previous observations from our laboratory and others (6, 15) have shown also that aminotransferase inhibitors reduce but do not prevent glutamine oxidation, further suggesting that some flux through GDH may occur. In preliminary experiments (data not shown), the potential toxicity of BCH to isolated intestinal cells was evaluated by quantifying CO_2

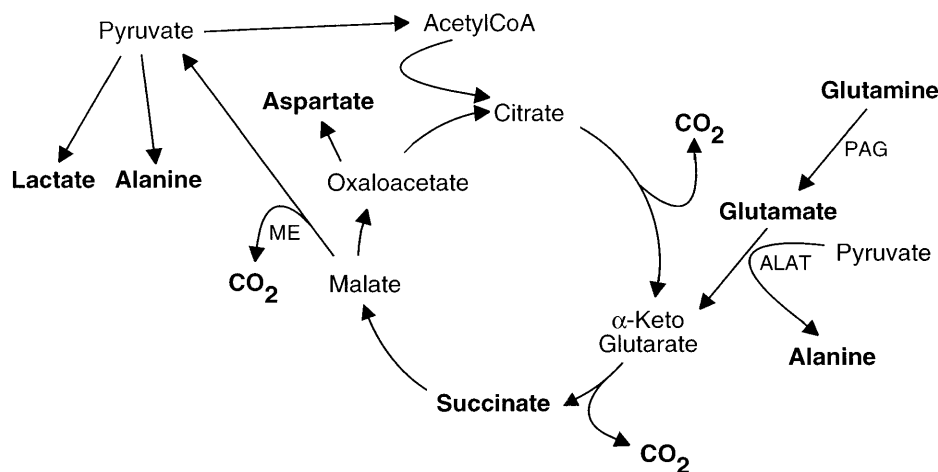


Fig. 1. Glutamine metabolic pathway. Intermediates and end products analyzed in these experiments are shown in boldface type. ALAT, alanine aminotransferase; ME, malic enzyme; PAG, phosphate-activated glutaminase.

production from glucose ($[U-^{14}C]$ glucose) and glutamine ($[U-^{14}C]$ glutamine). In *experiment 6*, cells were incubated with either $[1-^{14}C]$ glutamine or $[U-^{14}C]$ glutamine as tracers and 5 mmol/l glutamine, in either the absence or presence of 5 mmol/l BCH. Also, cells were incubated with $[U-^{14}C]$ glutamate and 5 mmol/l glutamate, in either the absence or presence of 5 mmol/l BCH. Cell suspensions were prepared from three animals, and each cell suspension was exposed to the six treatments.

The objective of *experiment 7* was to determine whether the relationship between glutamine entry into the TCA cycle and its fractional oxidation was constant along the length of the small intestine. Therefore, we isolated cells from the proximal and distal small intestine from four animals. Each cell preparation was then incubated with either $[1-^{14}C]$ glutamine or $[U-^{14}C]$ glutamine as tracers and with either 20 mmol/l glutamine or 20 mmol/l each of glutamine and glucose. Each of the four cell suspensions was exposed to all four treatments.

The objective of *experiment 8* was to determine whether the low rate of glutamate oxidation by intestinal epithelial cells is associated with low rates of entry into the TCA cycle. Cells from the proximal small intestine were incubated with 5 mmol/l glutamate, and CO_2 production from both $[U-^{14}C]$ glutamate and $[1-^{14}C]$ glutamate was measured to estimate glutamate oxidation to CO_2 and glutamate carbon entry into the TCA cycle, respectively. Cells were isolated from six animals, and each cell suspension was exposed to the two treatments.

In all experiments, values for each treatment were calculated as the mean of duplicate analyses for each cell preparation. Data are presented as means \pm SE. In most experiments, differences were determined using ANOVA techniques (repeated measures, one-way, and two-way ANOVA) and, when present, differences were identified using the Tukey's Studentized range test. Before undertaking ANOVA, variables were checked for equal variance and normal distribution of data to ensure suitability for that analysis. When there were significant interaction effects using ANOVA, follow-up tests were completed on the appropriate cell means rather than on the marginal means. In other experiments, pair-wise comparisons were made using the *t*-test, or the relationship between two variables was described using regression. Computer programs were used to perform computations (25). Results were considered statistically significant at $P < 0.05$.

RESULTS

Use of $[1-^{14}C]$ glutamate to estimate entry into TCA cycle of glutamine-derived glutamate. When both glutamine and glucose were present at 5 mmol/l, CO_2 was produced from $[U-^{14}C]$ glutamine at $\sim 50\%$ of the rate at which CO_2 was produced from $[1-^{14}C]$ glutamate (Table 1). Assuming that $[1-^{14}C]$ glutamate is a reliable predictor of the rate at which glutamine-derived glutamate enters the TCA cycle, then it can be estimated that 50% of the glutamine carbons entering the TCA cycle were oxidized to CO_2 . This proportion is in reasonable agreement with the A + T value of 0.46, obtained by using the succinate ratio method.

When glutamine was present at 0.3 mmol/l and glucose was present at 0.1 mmol/l, CO_2 production from $[U-^{14}C]$ glutamine exceeded by more than twofold the rate of glutamine carbon entry into the TCA cycle when determined using $[1-^{14}C]$ glutamate as tracer (Table 1). This result was unreasonable and also was not in agreement with the A + T value of 0.50 determined

Table 1. Influence of substrate concentration on fractional oxidation rates estimated using $[1-^{14}C]$ glutamate and $[U-^{14}C]$ glutamine vs. CO_2 ratio technique

Substrates	<i>n</i>	Oxidation to CO_2	Entry Into TCA Cycle	A + T
Glutamine (5 mmol/l) + glucose (5 mmol/l)	8	17.0 ± 1.2	34.0 ± 4.1	0.46 ± 0.06
Glutamine (0.3 mmol/l) + glucose (0.1 mmol/l)	4	6.37 ± 0.32	2.92 ± 0.39	0.50 ± 0.07

Values are means \pm SE. Data were taken from *experiment 1*. Values for oxidation to CO_2 using $[U-^{14}C]$ glutamine and entry into tricarboxylic acid (TCA) cycle using $[1-^{14}C]$ glutamate are given in μ mol carbon atoms \cdot g $^{-1}$ \cdot min $^{-1}$. Each cell suspension was exposed to 4 radioisotopes ($[U-^{14}C]$ glutamine, $[1-^{14}C]$ glutamate, $[1,4-^{14}C]$ succinate, and $[2,3-^{14}C]$ succinate). Values for A + T (given as a ratio) were calculated using data for CO_2 production from $[1,4-^{14}C]$ succinate and $[2,3-^{14}C]$ succinate; equation is given in MATERIALS AND METHODS (see footnote 1 for assumptions).

simultaneously. These results ruled out the possibility of using $[1-^{14}C]$ glutamate to measure TCA cycle entry of glutamine in concentrations < 5 mmol/l and thus also the $[1-^{14}C]$ glutamate and $[U-^{14}C]$ glutamate pair as tracers to calculate fractional oxidation rates for glutamine. In all subsequent experiments, $[U-^{14}C]$ glutamine and $[1-^{14}C]$ glutamine were used to measure glutamine oxidation to CO_2 and glutamine carbon entry into the TCA cycle, respectively.

Influence of glutamine concentration on glutamine oxidation and entry into TCA cycle. The production of $^{14}CO_2$ from $[U-^{14}C]$ glutamine increased with increasing glutamine concentration and reached a plateau at ~ 5 mmol/l glutamine (Fig. 2), suggesting saturation kinetics. A plot of $1/S$ vs. $1/v$ (where *S* refers to the substrate concentration and *v* refers to the rate of CO_2 production) gave a line with a regression coefficient > 0.999 . V_{max} was calculated to be 15.0μ mol $CO_2 \cdot g^{-1} \cdot min^{-1}$, and K_{ox} was calculated to be 0.4 mmol/l.

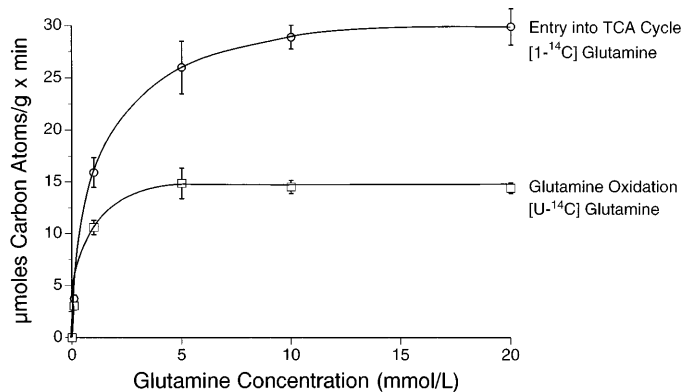


Fig. 2. Influence of glutamine concentration on glutamine oxidation ($^{14}CO_2$ from $[U-^{14}C]$ glutamine) and on the entry of glutamine carbon atoms into the tricarboxylic acid (TCA) cycle ($^{14}CO_2$ from $[1-^{14}C]$ glutamine). Cells, isolated from the jejunum of fed rats, were incubated with glutamine at concentrations ranging from 0.1 to 20.0 mmol/l and trace quantities of $[U-^{14}C]$ glutamine or $[1-^{14}C]$ glutamine. Production of $^{14}CO_2$ was used to calculate entry of glutamine carbon into the TCA cycle using equations presented in MATERIALS AND METHODS. Each data point represents mean \pm SE; $n = 3$. Data were taken from *experiment 2*.

The plot of $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ glutamine vs. glutamine concentration also suggested saturation kinetics (Fig. 2) and the plot of $1/S$ vs. $1/v$ gave a line with a regression coefficient >0.999 . Using the Lineweaver-Burk equation, V_{\max} was calculated to be $28.7 \mu\text{mol carbon atoms} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ and K_{ox} was calculated to be 0.7 mmol/l . In a separate experiment (data not shown), CO_2 production from $[1-^{14}\text{C}]$ glutamine was measured at low glutamine concentrations of 0.01 to 5 mmol/l . The regression coefficient of the Lineweaver-Burk plot was >0.999 . V_{\max} was calculated to be $30.2 \mu\text{mol carbon atoms} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, and K_{ox} was calculated to be 0.6 mmol/l .

Increasing the glutamine concentration of the incubation medium was associated with decreasing fractional oxidation rates of glutamine (Fig. 3). A linear and inverse relationship was observed between the rate of glutamine entry into the TCA cycle and the fractional oxidation rate of glutamine (Fig. 4). These two variables (glutamine entry rate and fractional oxidation of glutamine) had a significant correlation coefficient of -0.96 . Using regression analysis, the best-fit line had a slope of -0.012 and a y -intercept of 0.85 . These constants were subsequently used to predict fractional oxidation rates using TCA cycle entry data from other experiments to determine whether this relationship could be more generally applied.

In cells of the proximal small intestine, AOA reduced glutamine oxidation to CO_2 (using $[\text{U}-^{14}\text{C}]$ glutamine as tracer) by 51% when glutamine was the sole substrate, and reduced glutamine carbon entry into the TCA cycle (using $[1-^{14}\text{C}]$ glutamine as tracer) by 64% (Table 2). Consequently, the fractional oxidation of glutamine increased from 0.52 in the absence of AOA to 0.71 in the presence of AOA. The regression equation of glutamine

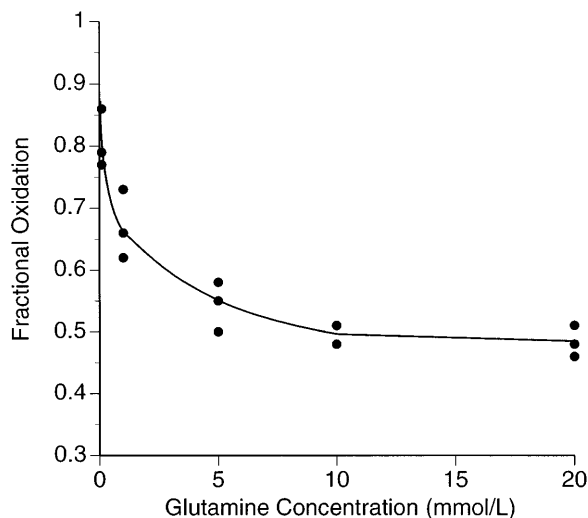


Fig. 3. Influence of glutamine concentration on fractional oxidation of glutamine. Cells, isolated from the jejunum of fed rats, were incubated with glutamine at concentrations of 0.1 , 1.0 , 5.0 , 10.0 , and 20.0 mmol/l and trace quantities of $[\text{U}-^{14}\text{C}]$ glutamine or $[1-^{14}\text{C}]$ glutamine. Production of $^{14}\text{CO}_2$ was measured, and fractional oxidation was calculated as $^{14}\text{CO}_2$ from $[\text{U}-^{14}\text{C}]$ glutamine/ $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glutamine. Each data point was calculated from the mean of duplicate analyses. Data were taken from *experiment 2*.

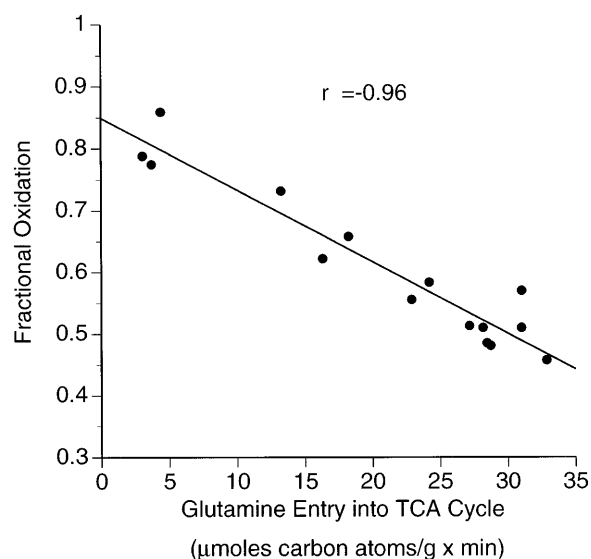


Fig. 4. Influence of the rate at which glutamine carbon enters the TCA cycle ($^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glutamine) on fractional oxidation of glutamine. Cells, isolated from the jejunum of fed rats, were incubated with glutamine at concentrations of 0.1 , 1.0 , 5.0 , 10.0 , and 20.0 mmol/l and trace quantities of $[\text{U}-^{14}\text{C}]$ glutamine or $[1-^{14}\text{C}]$ glutamine. Production of $^{14}\text{CO}_2$ was measured, and fractional oxidation was calculated as $^{14}\text{CO}_2$ from $[\text{U}-^{14}\text{C}]$ glutamine/ $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glutamine. Each data point was calculated from the mean of duplicate analyses. Data were taken from *experiment 2*. Linear regression was used to characterize the relationship between these variables (r , -0.96 ; slope, -0.012 ; y -intercept, 0.85).

entry into the TCA cycle vs. fractional oxidation rate, calculated for the data from *experiment 2* (Fig. 4), was used to predict fractional oxidation rates using the glutamine entry data for *experiment 4* (Table 2). The predicted fractional oxidation rate for the glutamine-only treatment (no AOA) was calculated to be 0.47 (vs. 0.52 as measured) and for the glutamine plus AOA treatment was calculated to be 0.71 (vs. 0.71 as measured). In the presence of glucose, AOA increased the fractional oxidation of glutamine from 0.53 to 0.75 (Table 2). Corresponding fractional oxidation rates, predicted from the data shown in Fig. 4, were 0.43 and 0.78 , respectively.

The incorporation of ^{14}C from $[\text{U}-^{14}\text{C}]$ glutamine into CO_2 was compared with ^{14}C incorporation into alanine, aspartate, lactate, and succinate since the TCA cycle is needed to convert glutamine into these metabolites. Including AOA in the medium reduced incorporation of glutamine carbon into each of these compounds, but the effects were most profound for alanine, succinate, and CO_2 . AOA decreased glutamine carbon incorporation into the non- CO_2 TCA cycle requiring metabolites by 66% , whereas the decrease in CO_2 production averaged 49% (Table 3). Label incorporation into glutamate was increased by 21% . Trace amounts of label were also found in proline, ornithine, and citrulline (data not shown), and the values obtained agree with those previously published (11).

BCH was not found (data not shown) to significantly influence glutamate oxidation to CO_2 ($[\text{U}-^{14}\text{C}]$ glutamate, 5 mmol/l glutamate), glutamate entry into the TCA cycle ($[1-^{14}\text{C}]$ glutamate), or fractional oxidation of

Table 2. Influence of AOA on glutamine entry into TCA cycle and oxidation to CO₂ by jejunal epithelial cells

	Glutamine		Glutamine + Glucose		P for FStatistic		
	-AOA	+AOA	-AOA	+AOA	Sub	Inh	Sub × Inh
CO ₂ production, μmol CO ₂ · g ⁻¹ · min ⁻¹	16.6 ± 1.5*	8.19 ± 0.6†	19.7 ± 3.4*	4.55 ± 0.6†	0.83	0.009	0.040
Glutamine carbon entry, μmol C atoms · g ⁻¹ · min ⁻¹	32.5 ± 4.6*	11.8 ± 1.5†	36.2 ± 3.6*	6.17 ± 0.6†	0.56	0.004	0.017
Fractional oxidation							
Measured	0.52 ± 0.04	0.71 ± 0.06	0.53 ± 0.05	0.75 ± 0.08	0.77	0.001‡	0.65
Predicted	0.47	0.71	0.43	0.78			

Values are means ± SE; *n* = 4. Because of the significant interaction between substrate (Sub) and inhibitor (Inh), differences were determined among the 4 treatment means. Data were taken from *experiment 4*. Substrates were present at 5 mmol/l. [U-¹⁴C]glutamine served as tracer. Equation for measured fractional oxidation is given in MATERIALS AND METHODS. AOA, (aminoxy)acetic acid. Predicted fractional oxidation from regression equation calculated for data in Fig. 4, where $y = ax + b$. *x*, Measured rate of glutamine entry into TCA cycle; *y*, predicted fractional oxidation; *a*, slope (-0.012); *b*, *y*-intercept (0.85). *† Significantly different (*P* < 0.05) using Tukey's follow-up test. ‡ Pooled mean value was significantly lower for -AOA (without AOA) than +AOA (with AOA) treatments (0.53 vs. 0.73, respectively).

glutamate (glutamate oxidation/glutamate carbon entry into the TCA cycle). Also, BCH was found to not significantly influence glutamine oxidation to CO₂, glutamine entry into the TCA cycle, or fractional oxidation of glutamine. Consequently, we were unable to use this approach to study further the relationship between glutamate entry into the TCA cycle and fractional oxidation rates.

Relationship between glutamine entry into TCA cycle and fractional oxidation: proximal vs. distal small intestine. In the presence of either glutamine alone or glutamine plus glucose, CO₂ production from [U-¹⁴C]glutamine was significantly higher for cells from the proximal than distal small intestine (Table 4). Similarly, glutamine carbon entry, determined using [1-¹⁴C]glutamine, was significantly higher in the proximal than in the distal segment for both substrate treatments. The measured fractional oxidation values were not significantly different for the two segments.

The regression equation of glutamine entry into the TCA cycle vs. fractional oxidation rate (Fig. 4) was used to predict fractional oxidation rates for the data from *experiment 7*. The predicted fractional oxidation rates for data taken from cells of the proximal segment agreed within 15% with the measured fractional oxidation values (Table 4). Fractional oxidation rates measured for cells taken from the distal small intestine were not accurately predicted by this equation, however, as measured values differed from predicted values by >40%.

Fractional oxidation of glutamate determined using [¹⁴C]glutamate tracers. The fractional oxidation rate for glutamate averaged 0.77 in cells of the proximal small

intestine when glutamate was provided at 5 mmol/l (Table 5). The regression equation of glutamine entry into the TCA cycle vs. fractional oxidation rate that was calculated for the data from *experiment 2* (Fig. 4) was used to predict fractional oxidation rates for these glutamate data (Table 5). The predicted fractional oxidation rate (0.74) for glutamate differed by <4% from the measured fractional oxidation rate (0.77).

DISCUSSION

Use of [U-¹⁴C]glutamine and [1-¹⁴C]glutamine to measure fractional oxidation of glutamine. For several reasons, it appears that entry of glutamine-derived glutamate into the TCA cycle and fractional oxidation of glutamine can be accurately measured using [U-¹⁴C]glutamine and [1-¹⁴C]glutamine as tracers. The observations using [¹⁴C]glutamine tracers provided data that were intuitively correct, as ¹⁴CO₂ production from both [1-¹⁴C]glutamine and [U-¹⁴C]glutamine showed saturation kinetics with increasing glutamine concentrations (Fig. 2), and fractional oxidation never exceeded the theoretical maximum of 100%. Furthermore, the value of 27.7 μmol C atoms · g⁻¹ · min⁻¹, calculated as the total of the five TCA cycle-requiring metabolites (alanine, aspartate, CO₂, lactate, and succinate; Table 3) approximates the value of 32.5 μmol C atoms · g⁻¹ · min⁻¹, calculated for glutamine carbon entry determined using [1-¹⁴C]glutamine (Table 2). Fractional oxidation values decreased with increasing concentrations of glutamine (Fig. 3), supporting the trend observed previously using the CO₂ ratios approach (10).

It was surprising to observe that fractional oxidation of glutamine could not be accurately measured using [1-¹⁴C]glutamate and [U-¹⁴C]glutamate as tracers. The CO₂ ratios technique, pioneered many years ago (27) and universally utilized, is based on the principle that pathway intermediates can be used to quantify the extent to which a substrate is oxidized in the TCA cycle to CO₂. We reasoned that the same approach could be used to assess the relative oxidation of glutamine but with isotopes of glutamate as tracers. This proved not to be the case, suggesting that there are multiple intracellular glutamate pools and that glutamine concentration influences the relative size and specific activity of these pools. Glutamine and glutamate are

Table 3. Influence of AOA on incorporation of glutamine carbon into metabolites

Metabolite	-AOA	+AOA	%Change
Alanine	7.86 ± 0.18	1.65 ± 0.11	
Aspartate	1.27 ± 0.27	0.96 ± 0.21	
Lactate	1.97 ± 0.05	1.40 ± 0.04	
Succinate	0.69 ± 0.03	0.07 ± 0.01	
Total	11.9	4.08	-66
CO ₂	15.8 ± 0.46	8.00 ± 0.16	-49
Glutamate	13.2 ± 1.1	16.0 ± 0.8	21

Values are means ± SE; *n* = 3. Data were taken from *experiment 5*. Values for -AOA and +AOA are given in μmol glutamine carbon atoms · g⁻¹ · min⁻¹. Glutamine was present at 5 mmol/l. Total includes values for alanine, aspartate, lactate, and succinate.

Table 4. Influence of glucose on glutamine entry and oxidation in cells of proximal vs. distal small intestine

Substrate	Glutamine		Glutamine + Glucose		P for F-Statistic		
	Proximal	Distal	Proximal	Distal	Sub	Seg	Sub × Seg
CO ₂ production, μmol CO ₂ · g ⁻¹ · min ⁻¹	16.1 ± 1.7	7.55 ± 0.9	18.1 ± 0.6	7.11 ± 1.1	0.398	0.005§	0.10
Glutamine carbon entry, μmol carbon atoms · g ⁻¹ · min ⁻¹	34.0 ± 3.2*	16.7 ± 1.5†	39.4 ± 3.2‡	17.1 ± 1.7†	0.006	0.002	0.003
Fractional oxidation							
Measured	0.47 ± 0.01	0.45 ± 0.05	0.46 ± 0.03	0.42 ± 0.07	0.61	0.57	0.45
Predicted	0.46	0.65	0.40	0.65			

Values are means ± SE; *n* = 3. Because the interaction between substrate (Sub) and segment (Seg) was statistically significant, differences were determined among the 4 treatment means. Data were taken from *experiment 7*. Substrates were present at 20 mmol/l. See Table 2 legend for explanation of measured and predicted fractional oxidation. *†‡Significantly different (*P* < 0.05) using Tukey's follow-up test. §Pooled mean was significantly higher for proximal vs. distal segment (17.1 vs. 7.33, respectively).

known to be transported across enterocyte plasma and subcellular membranes via different mechanisms and at different rates (6, 20, 29). Although care was taken to ensure that the ratio of exogenously provided glutamine to glutamate was constant for the [U-¹⁴C]glutamate and [1-¹⁴C]glutamate treatments, it appears that transport or other factors caused differences in the specific activities of intermediates in key intracellular compartments or metabolic pools. Isotopes of glutamate could not be used to measure the fractional oxidation of glutamine.

Fractional oxidation rates for glutamine, using [U-¹⁴C]glutamine and [1-¹⁴C]glutamine as tracers, were in good agreement with measurements made using the principles of the CO₂ ratios technique.¹ When both glutamine and glucose were present at 5 mmol/l, the

value for A + T was 0.46 (Table 1) and, using [U-¹⁴C]glutamine and [1-¹⁴C]glutamine as tracers, fractional oxidation of glutamine was found to be 0.53 (Table 2). These values are within 15% and indicate close agreement between these two quite different approaches to determining the extent to which the glutamine molecule is oxidized to CO₂ by jejunal epithelial cells. The fractional oxidation technique that utilizes [¹⁴C]glutamine tracers is a more direct approach, however, and avoids having to make assumptions regarding the homogeneity of the pools of intermediates originating from different substrates.

As further evidence that [U-¹⁴C]glutamine and [1-¹⁴C]glutamine data can be used to measure fractional oxidation of glutamine, an experiment was conducted to directly measure the incorporation of glutamine carbon into metabolites of the TCA cycle. This experiment was conducted in the absence and presence of AOA, since AOA was found to reduce by ~50% the entry of glutamine carbon into the TCA cycle and to increase fractional oxidation of glutamine (Table 2). Overall, AOA reduced CO₂ production by 49% and reduced non-CO₂ metabolite production by 66% (Table 3). The greater influence of AOA on non-CO₂ metabolites vs. CO₂ would be expected, based on the increased fractional oxidation of glutamine observed using the [U-¹⁴C]glutamine/[1-¹⁴C]glutamine approach (Table 2). This further supports the use of [¹⁴C]glutamine tracers for calculating fractional oxidation.

¹ The CO₂ ratio is defined as the steady-state production of ¹⁴CO₂ from [1-¹⁴C]acetate or [1,4-¹⁴C]succinate divided by that from [2-¹⁴C]acetate or [2,3-¹⁴C]succinate (13, 27). While [2-¹⁴C]acetate or [2,3-¹⁴C]succinate must remain in the cycle for a complete turn before any ¹⁴CO₂ is released, [1-¹⁴C]acetate or [1,4-¹⁴C]succinate is oxidized to ¹⁴CO₂ during the first turn of the cycle. Thus more ¹⁴CO₂ can be produced from [1,4-¹⁴C]succinate than from [2,3-¹⁴C]succinate when efflux of TCA cycle intermediates occurs. The probability that a compound is oxidized rather than incorporated into a product after its efflux from the TCA cycle can be predicted by calculating the variable previously referred to as A + T (13), where A is the probability that carbon entering the TCA cycle will be incorporated into citrate via acetyl-CoA, and T is the probability that carbon will be incorporated into citrate via oxaloacetate. The variable A + T thus equals the probability that carbon will complete one turn of the TCA cycle by either remaining in the cycle (path T) or leaving as a four-carbon fragment and reentering via acetyl-CoA (path A). Thus a higher A + T value represents a greater probability of complete oxidation and lower efflux of TCA cycle intermediates. A + T predicts the probability that carbon atoms entering the TCA cycle will remain in the TCA cycle for one complete turn and, as such, is an index of oxidation of the substrate molecule. The theoretical basis for use of ¹⁴CO₂ production ratios to estimate flux into the TCA cycle and net oxidation of a substrate is derived from classic work on steady-state TCA cycle labeling patterns (27). This approach requires making the following assumptions (13): 1) the system is in metabolic and isotopic steady state when data are collected so that the specific activity ratios among the citrate carbons are constant, 2) sources of radioactivity may be varied without altering the chemical composition of the medium, 3) the specific activity of any pool of TCA cycle intermediates cannot be determined by direct experimental measurement, 4) TCA cycle metabolism is not compartmentalized in a manner that causes the fate of intermediates to be dependent on the precursors from which they were formed, and 5) the system is not compartmentalized in such a way that the tracer (acetate or succinate) and substrate (glutamine) are metabolized by separate types of cells or mitochondria. A study by Mallet et al. (18) in rat enterocytes provided evidence substantiating these assumptions.

Table 5. Fractional oxidation of glutamate using [¹⁴C]glutamate tracers, and comparison to fractional oxidation predicted from regression analysis using glutamine substrate and tracers

	Glutamate
CO ₂ production, μmol CO ₂ · g ⁻¹ · min ⁻¹	7.04 ± 0.39
Glutamate carbon entry, μmol carbon atoms · g ⁻¹ · min ⁻¹	9.18 ± 0.43
Fractional oxidation	
Measured	0.77 ± 0.04
Predicted	0.74 ± 0.01

Values are means ± SE; *n* = 6. Data were taken from *experiment 8*. Glutamate was used at a concentration of 5 mmol/l. [U-¹⁴C]glutamate was used as tracer for CO₂ production, and [1-¹⁴C]glutamate was used as tracer for glutamate carbon entry. Production of ¹⁴CO₂ was measured, and measured fractional oxidation was calculated as ¹⁴CO₂ from [U-¹⁴C]glutamate/¹⁴CO₂ from [1-¹⁴C]glutamate. See Table 2 for explanation of predicted fractional oxidation.

Factors influencing fractional oxidation rate of glutamine. Fractional oxidation of glutamine was influenced by the rate at which glutamine carbon entered the TCA cycle. At low rates of entry, the glutamine molecule may be 80–90% oxidized to CO₂ (Fig. 4). At high rates of entry there may be little, if any, reentry of carbon into the TCA cycle as the molecule appears under these conditions to be only 40% oxidized.

Entry of glutamine carbon into the TCA cycle was markedly influenced by changes in the glutamine concentration of the medium (Fig. 2). The K_{ox} for glutamine carbon entry into the TCA cycle was calculated to be 0.6–0.7 mmol/l, and this function was saturated at 5 mmol/l. Only at near saturating concentrations of glutamine can glutamine carbon be substantially incorporated into three-carbon intermediates, including lactate and alanine (26). The K_{ox} of 0.6–0.7 mmol/l for CO₂ production from [1-¹⁴C]glutamine was somewhat higher than the K_{ox} of 0.4 (see RESULTS) or 0.45 mmol/l (14) for production of CO₂ from [U-¹⁴C]glutamine. These values suggest that in fed rats, in which the normal arterial glutamine concentration is ~0.50 mmol/l (31), glutamine will be oxidized to CO₂ at ~50% of the maximal oxidation rate, whereas entry of glutamine carbon will be <50% of maximal entry rates. Glutamine may enter the TCA cycle at maximum rates and may be maximally oxidized to CO₂, however, if luminal glutamine concentrations increase to 5 mmol/l or more after meal feeding. The glutamine plus asparagine concentration in jejunal contents has been reported to be 1.2 mmol/l after consumption of a high protein meal (1), but less destructive hydrolysis techniques would need to be employed to determine with certainty luminal glutamine concentrations.

The maximum rate of entry of glutamine into the TCA cycle was twofold higher than the rate at which CO₂ was produced from the glutamine molecule (Fig. 2). This is comparable to the ratio of 2.5:1 observed previously (26). These ratios indicate that, under conditions when the substrate is present at concentrations that saturate the pathways, each glutamine molecule that enters the TCA cycle as α-ketoglutarate undergoes two decarboxylations to yield CO₂. The remaining carbons were found previously in three carbon intermediates, such as alanine and lactate (26). Our results (Table 3) support and expand on this previous observation, because we show that glutamine availability influences glutamine carbon entry into the TCA cycle, which, in turn, influences the fractional oxidation of the glutamine molecule. Thus glutamine availability influences the metabolic fate of glutamine carbon entering the TCA cycle.

The presence of glucose stimulated glutamine entry into the TCA cycle (Tables 2 and 4). Pyruvate-producing compounds other than glucose could also be expected to increase glutamine entry into the TCA cycle since it is thought that glucose stimulates TCA cycle entry by providing a pyruvate source for transamination with glutamine-derived glutamate (15). Accordingly, compounds that would inhibit transaminase activity would be expected to reduce the rate of entry into the TCA

cycle and increase fractional oxidation. This is supported by our observations that AOA decreased entry, increased fractional oxidation (Table 2), and decreased incorporation of glutamine into CO₂ to a lesser extent than into non-CO₂ metabolites of the TCA cycle (Table 3). The degree of change agreed very well with the predicted fractional oxidation rate, based on the relationship between these two variables that was established in a previous experiment (Fig. 4). Our attempt to stimulate glutamine carbon entry into the TCA cycle with the purported stimulator of GDH, BCH, was not successful, however (see RESULTS). Our results suggest that BCH did not stimulate GDH activity in intestinal epithelial cells as it has been shown to do in other systems (7, 17, 24).

Metabolism of glutamate. The current studies expand our understanding of glutamate metabolism in the following way. When glutamine or glutamate is present in cell suspensions at equimolar concentrations (5 mmol/l), glutamate carbon enters the TCA cycle at approximately one-third the rate of glutamine carbon (Table 2 vs. Table 5). Because glutamate requires only to be transported to the site of its conversion to α-ketoglutarate (Fig. 1), whereas glutamine must be both transported and deamidated, one might expect that glutamate carbon would enter the TCA cycle at a greater rate than glutamine carbon. This was not observed here (Tables 2 and 5) or elsewhere (15, 29). The lower rate of entry of glutamate carbon into the TCA cycle may be due to the slower absorption rate into intestinal cells or mitochondria of glutamate compared with glutamine (6, 29). At 5 mmol/l, glutamate carbon also was more completely oxidized than glutamine carbon, as indicated by a higher fractional oxidation value of 0.77 for glutamate (Table 5) vs. 0.52 for glutamine (Table 2). When the regression equation of glutamine carbon entry vs. fractional oxidation (Fig. 4) was used to predict the fractional oxidation rate of glutamate from the glutamate carbon entry data reported here (Table 5), the measured and predicted values for fractional oxidation were in good agreement. This suggests that the high fractional oxidation rate for glutamate vs. glutamine is probably a consequence of the lower rate at which glutamate carbon enters the TCA cycle in intestinal cells. These observations with glutamate, together with our observations with glutamine, support a general conclusion that the fractional oxidation within the TCA cycle of these molecules is determined by their rate of entry into the TCA cycle. Entry rate, in turn, is influenced by their extracellular concentration, their rate of transport into the cell, and the rates at which they are metabolized to α-ketoglutarate.

When glutamine is the sole substrate, in addition to entry into the TCA cycle, glutamine-derived glutamate can also accumulate in the cells or be secreted back into the medium. Even though intracellular glutamate cannot be distinguished from labeled glutamate released into the medium, the total nonglutamine radioactivity can serve as a measure of glutamine processed through glutaminase. In *experiment 5*, labeled glutamate (see

RESULTS) amounted to an additional 41% above the value for glutamine entry into the TCA cycle (using Table 2 data). At a substrate concentration of 5 mmol/l (saturating conditions), the capacity of glutaminase to generate glutamate exceeded the capacity of the TCA cycle to process it further. Inhibiting the transaminase removal of glutamate by using AOA reduced the TCA cycle entry to 36% of the uninhibited level but produced only a 21% increase in [¹⁴C]glutamate. Total glutamine deamidation was reduced to 60% of the uninhibited level, reflecting a possible inhibitory effect of intracellular glutamate accumulation on the action of glutaminase.

Glutamine metabolism in proximal vs. distal small intestine. Cells from the proximal small intestine showed a significantly greater rate of CO₂ production from glutamine than did cells from the distal segment (Table 4). These results are consistent with previous observations (15). Also, the rate of glutamine carbon entry into the TCA cycle was approximately twofold higher for cells of the proximal vs. distal small intestine, resulting in similar values for fractional oxidation (Table 4). The measured values for fractional oxidation were not in good agreement with the values predicted using the regression equation of glutamine entry vs. fractional oxidation (Fig. 4) generated using cells of the proximal small intestine. These few results in the distal small intestine suggest that the relationship between fractional oxidation and glutamine entry rates may differ substantially along the length of the small intestine.

In conclusion, our results suggest that the availability of glutamine has a marked influence on the metabolic fate of its carbons that enter the TCA cycle. Increasing entry of glutamine into the TCA cycle was associated with decreased fractional oxidation rates of glutamine under several conditions, resulting in greater incorporation into synthetic products such as lactate, alanine, and succinate. It can be expected that there would be increased incorporation also into minor, but physiologically important, synthetic products, including lipids or other compounds using TCA cycle-derived precursors. These results will have clinical implications if such precursors are essential for repair or replacement of diseased or damaged intestinal tissues.

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