Adaptative increase of ornithine production and decrease of ammonia metabolism in rat colonocytes after hyperproteic diet ingestion

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Mouillé, Béatrice, Véronique Robert, and François Blachier. Adaptative increase of ornithine production and decrease of ammonia metabolism in rat colonocytes after hyperproteic diet ingestion. Am J Physiol Gastrointest Liver Physiol 287: G344–G351, 2004. First published April 2, 2004; 10.1152/ajpgi.00445.2003.—Chronic high-protein diet consumption leads to increased concentrations of NH₄⁺/NH₃ in the colon lumen. We asked whether this increase has consequences on colonic epithelial cell metabolism. Rats were fed isocaloric diets containing 20 (P20) or 58% (P58) casein as the protein source for 7 days. NH₄⁺/NH₃ concentration in the colonic lumen and in the colonic vein blood as well as ammonia metabolism by isolated surface colonic epithelial cells was determined. After 2 days of consumption of the P58 diet, marked increases of luminal and colonic vein blood NH₄⁺/NH₃ concentrations were recorded when compared with the values obtained in the P20 group. Colonocytes recovered from the P58 group were characterized at that time and thereafter by an increased capacity for L-ornithine and urea production through arginase (P < 0.05). L-Ornithine was mostly used in the presence of NH₄Cl for the synthesis of the metabolic end product L-citrulline. After 7 days of the P58 diet consumption, however, the ammonia metabolism into L-citrulline was found lower (P < 0.01) when compared with the values measured in the colonocytes recovered from the P20 group despite any decrease in the related enzymatic activities (i.e., carbamoyl-phosphate synthetase I and ornithine carbamoyl transferase). This decrease was found to coincide with a return of blood NH₄⁺/NH₃ concentration in colonic portal blood to values close to the one recorded in the P20 group. In response to increased NH₄⁺/NH₃ concentration in the colon, the increased capacity of the colonocytes to synthesize L-ornithine is likely to correspond to an elevated L-ornithine requirement for the elimination of excessive blood ammonia in the liver urea cycle. Moreover, in the presence of NH₄Cl, colonocytes diminished their synthesis capacity of L-citrulline from L-ornithine, allowing a lower cellular utilization of this latter amino acid. These results are discussed in relationship with an adaptative process that would be related to both interorgan metabolism and to the role of the colonic epithelium as a first line of defense toward luminal NH₄⁺/NH₃ concentrations.

Colonic epithelial cells

THE DIETARY PROTEIN CONSUMPTION level is vastly different according to food availability and cultural dietary habits. In Western Europe and in the United States for example, protein consumption averages ~1.5–2 times the recommended intakes (i.e., 0.75–0.83 g · kg⁻¹ · day⁻¹) (13, 33) and as much as four times these values in slimming high-protein diets. Although there are many studies on the metabolic adaptations involved in response to increased protein ingestion (23, 34), the physiological consequences of high-protein diet consumption are still poorly understood, and the maximal nonleterious level of intake is still to be defined (17, 23).

In people consuming a Western-type diet, Chacko and Cummings (8) have reported that up to 12 g protein may escape digestion. Thus a high dietary protein level may exceed the digestive capacities and amino acid/oligopeptide absorptive capacities of the small intestine (15), resulting in an increased protein influx in the large intestine lumen. Proteins escaping digestion, including those of endogenous origin (e.g., digestive enzymes and sloughed mucosal cells), reach the large intestine, where they undergo catabolism by the colonic flora, leading to the release in the colon lumen of a number of potentially harmful compounds including phenols, indoles, amines, sulfide, and ammonia (21). NH₄⁺/NH₃, which is the major nitrogenous end product of bacterial activities, is mainly produced through deamination of amino acids and, to a lesser extent, by urea hydrolysis in the large bowel (38, 43). The luminal NH₄⁺/NH₃ concentration is primarily the net result of the bacterial NH₄⁺/NH₃ production, assimilation for bacterial de novo protein synthesis and large intestine absorption. The highest concentration of NH₄⁺/NH₃ in the body is found in the large intestine lumen. Indeed, NH₄⁺/NH₃ concentrations in the large intestine content, which can vary greatly according to the amount of dietary proteins, average 20–70 mM in rats (19) and 3–44 mM in fecal dialysates of humans (44). An increase in the amount of dietary proteins leads to a rise in human fecal NH₄⁺/NH₃ concentration from 15 to 30 mM (12, 14). In healthy individuals, NH₄⁺/NH₃ is transported to the liver by the portal vein, where it is mostly removed through urea synthesis in the hepatic ornithine cycle (22) and incorporation of NH₄⁺ into L-glutamate leading to L-glutamine synthesis (16). It has been demonstrated that activities of the five urea-cycle enzymes in the rat liver are increased in response to increased dietary protein intake (26, 34) as a result of pretranslational regulations (27). An increase in NH₄⁺/NH₃ concentration in the systemic circulation is detrimental, because this compound is toxic to the central nervous system (32). Furthermore, it has been proposed that the transient depressed food intake and the consequent reduction in body weight gain during high-protein diet consumption may be partly due to a transient increase in plasma NH₄⁺/NH₃ concentration resulting in increased entry into the central nervous system (29, 35).

We have previously demonstrated that in normal rat colonocytes, the metabolism of ammonia leads to L-citrulline production through the sequential action of two enzymes. Carbamoyl phosphate (carbamoyl-P) synthesized by the intramitochon-
drial carbamoyl-P synthetase I (CPSI) is channeled through ornithine carbamoyltransferase (OCT), which combines this metabolite with L-ornithine to form L-citrulline. Incorporation of ammonia into L-citrulline by the colon epithelial cells may correspond to a first line of defense against luminal NH4+/NH3 entry into the bloodstream (25).

In this context, the purpose of this study was to determine whether ammonia detoxification metabolism by colonic epithelial cells would adapt to a hyperproteic diet that increases the NH4+/NH3 concentration in the colonic lumen.

**MATERIALS AND METHODS**

*Chemicals.* L-[guanido-14C]arginine, L-[U-14C]arginine, L-[ureido-13C]citrulline, L-[1-14C]ornithine, L-[U-14C]ornithine, and NaH14CO3 were purchased from Amersham (Little Chalfont, UK) and from New England Nuclear (Boston, MA). Hyaluronidase, ornithine carbamoyltransferase, argininosuccinate lyase, inorganic pyrophosphate, and carbamoylphosphate were from Sigma (St Louis, MO). All enzymes and coenzymes used for enzymatic assays were from Roche (Meylan, France). D,L-α-difluoromethylornithine (DFMO) was kindly provided by the Marion Merrel Dow Research Institute (Strasbourg, France). Diets were prepared by the Atelier de Preparation des Aliments Experimentaux, Institut National de la Recherche Agronomique (Jouy-en-Josas, France).

*Animals.* Two-month-old Fisher male rats, weighing 207 ± 3 g, were assigned to two different groups. They were housed in individual cages and fed one of the two semipurified diets containing two concentrations of casein (see the composition of the diets in Table 1). These diets were compounded so that their energy contents were similar in the two experimental diets. The diets were also equalized in minerals, vitamins, and fat. The animals had free access to the food and tap water. Individual body weights were recorded daily.

Two or four days or one week after the initiation of the experiments, the rats were intraperitoneally anesthetized between 10:00 and 11:00 AM with pentobarbital sodium (40 mg/kg body wt). All aspects of the present protocol conformed to the requirements of the laboratory’s approval for animal research (1987 regulation). Fatty tissue was removed to expose the right colonic vein, and blood was collected with a heparinized syringe. The pH of the cecal and colonic contents was measured using a penetration electrode (Fisher Bioblock Scientific, Illkirch, France).

The cecum and the large bowel were then removed, and the colon was divided into three parts representing the proximal, median, and distal colon. Colonic and cecal contents of individual rats were collected by expulsion and stored at −80°C until processing. For colon epithelial cell isolation (see Colon epithelial cell isolation and incubation), the colon was entirely removed.

**Ammonia measurement.** NH4+/NH3 concentrations in blood samples (500 μl) recovered from the right colonic vein obtained before killing were determined. Deproteinized blood, treated with ice-cold perchloric acid (PCA; final concentration: 4%), was neutralized, and the ammonium concentration was assayed, from a standard reference curve made with different concentrations of ammonium chloride, by a specific enzymatic method (1) using a Uvikon 860 recording spectrophotometer (Kontron, Les Ulis, France).

NH4+/NH3 concentrations were measured in colonic luminal contents (500 μl). They were first deproteinized [with ice-cold PCA (final concentration: 4%)], then neutralized, and these suspensions were then used to determine ammonium concentrations using the same specific enzymatic method as described earlier.

**Colon epithelial cell isolation and incubation.** Colonic epithelial cells (colonocytes) were obtained from isolated colons as described (9). Briefly, the colons were removed and flushed clean with NaCl solution (9 g/l) and then with a Ca2+- and Mg2+-free Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 10 mM HEPES, 5 mM dithioretil, and 2.5 g/l bovine serum albumin and equilibrated against a mixture of O2 and CO2 (19:1, vol/vol). Then, the colons were perfused for 20 min at 37°C with the same buffer containing 5 mM EDTA. The luminal fluid was collected, and the cell fraction was incubated for 15 min at 37°C using the same buffer except for the absence of EDTA and the presence of hyaluronidase (4 g/l). The isolated cells were then washed twice and resuspended in a Krebs-Henseleit bicarbonate-buffered medium (pH 7.4) saturated with a mixture of O2 and CO2 (19:1, vol/vol) containing (in mM) 120 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.2 Na2SO4, 10 HEPES, 1.3 CaCl2, 2 MgCl2, and 25 NaHCO3, and enriched with 10 g/l bovine serum albumin (incubation medium). The colonocytes were counted on a hemocytometer. Previous work (9) has determined that the isolation procedure used in this study led to the recovery of absorbing colonocytes, which represent at least 87% of the isolated cells. Colonocyte viability was checked by measuring the percentage of the cytosolic enzyme lactate dehydrogenase activity released in the extracellular medium (7). Freshly isolated colonocytes were used immediately for metabolic activity measurement. Cell viability was never <80% at the onset of incubation. Incubated cells were incubated in 120 μl incubation medium containing the radiolabeled precursors. At the end of the incubation, the samples were kept at −80°C until the HPLC procedure (see HPLC).

**Enzymatic activities.** Isolated colonocytes were kept at −80°C before measurement of enzymatic activities. They were homogenized by sonication at 4°C. Arginase activity was measured at 37°C in 100 μl of a Tris·HCL buffer (100 mM, pH 7.2) containing 2 mM MnCl2 and increasing concentrations of L-[guanido-14C]arginine. Incubation was stopped with PCA (final concentration: 2%), and the samples were then separated from 1-arginine by HPLC (see HPLC). Cytosolic and particulate arginase activities were obtained from colonocytes homogenized at 4°C by mechanical disruption and sonication (5 × 10 s in a Tris-HCl buffer (100 mM, pH 7.2). The homogenate was then centrifuged at 4°C for 90 min at 100,000 g. The pellet (particulate arginase activity) was then rehomogenized at 4°C

Table 1. Composition of the experimental diets P20 and P58

<table>
<thead>
<tr>
<th></th>
<th>Control Protein Diet (P20)</th>
<th>High Protein Diet (P58)</th>
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<tbody>
<tr>
<td>Metabolizable energy, kJ/g</td>
<td>37.13</td>
<td>37.17</td>
</tr>
<tr>
<td>Dry matter, g/kg</td>
<td>31.05</td>
<td>30.00</td>
</tr>
<tr>
<td>Casein*</td>
<td>20.0</td>
<td>58.00</td>
</tr>
<tr>
<td>Cornstarch*</td>
<td>23.35</td>
<td>160.0</td>
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<tr>
<td>Sucrose</td>
<td>20.0</td>
<td>38.5</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>20.0</td>
<td>70.0</td>
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<td>Colza oil</td>
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<td>25.0</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>n-Methionine</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>45.0</td>
<td>45.0</td>
</tr>
</tbody>
</table>

⁎HIC01 casein, UCCS, Surgeres, France. ⑨Cornstarch, Cerestar, Saint-Maur, France. ⑩α-Cellulose, Durieux, Mame-la-Vallée, France. ⊳Colza and Groundnut oil, Bailly,aultay-sous-Bois, France. ①n-methionine, Central Soya, Trappes, France. ②Vitamin mix CV102, Central Soya, Trappes, France: Vitamin A (500 IU); Vitamin E (2500 IU); Vitamin K (100 IU); thiamin HCl, 1.0 g/kg; riboflavin B2, 1.0 g/kg; nicotinic acid, 4.5 g/kg; D-calcium pantothenate, 3.0 g/kg; pyridoxine HCl, 1.0 g/kg; inositol, 5.0 g/kg; p-biotin, 0.02 g/kg; folic acid, 0.2 g/kg; cyanocobalamin, 0.00135 g/kg; ascorbic acid, 10 g/kg; para amino benzoic acid, 5.0 g/kg; choline chloride, 75.0 g/kg; choline chloride, 150.0 g/kg. ③Mineral mix, Prolabo Merck, Dormstadt, Germany (in g/kg mineral mixture): CaHPO4, 2H2O, 380.0; KH2PO4, 240.0; CaCO3, 180.0; MgSO4, 7H2O, 90.0; NaCl, 69.0; MgO, 20.0; FeSO4·7H2O, 8.6; ZnSO4·H2O, 5.0; MnSO4·H2O, 5.0; CaSO4·2H2O, 1.0; NaF, 0.8; Cr2O3 (SO4), 12H2O, 0.5; KI, 0.01; (NH4)2MoO4, 4H2O, 0.02; CoCl2·6H2O, 0.02; Na2SeO3, 0.02. ④Isotonic diet-fed rats with 20% casein; P58, isotonic diet-fed rats with 58% casein.
10 s) in 300 μl of the Tris-HCl buffer. Arginase activities were finally assayed using the particulate fraction and 100,000 g cytosolic supernatant (cytosolic arginase activity) in the presence of 20 mM L-[guanido-14C]arginine. OCT activity was measured at 37°C in 100 μl of a Tris-HCl buffer (50 mM, pH 8.3) containing 0.5 mM carbamoylphosphate and increasing concentrations of L-[U-14C]ornithine. The reaction was halted at –80°C and l-citrulline was separated from L-ornithine by HPLC (see HPLC). CPSI activity was measured on the cells previously sonicated in the presence of 20 μl of a cocktail of protease inhibitors (0.4 mg/ml 4-(2-aminooethyl)benzenesulfonfyl fluoride, 5 mg/ml EDTA-Na2, 1 μg/ml leupeptin, and 1 μg/ml pepstatin). The enzyme assay was then performed at 37°C for 60 min in 100 μl of a Tris-HCl buffer (50 mM, pH 8.3) containing (in mM) 15 NaH14CO3, 20 ATP, 2 N-acetylglutamate, 10 MgSO4, 10 NH4Cl, and 10 l-ornithine and 15 U OCT. The reaction was halted at –80°C, and l-citrulline was separated from NaHCO3 by HPLC (see HPLC). Argininosuccinate synthase (ASS) was measured at 37°C in 100 μl of a Tris-HCl buffer (10 mM, pH 7.5) in the presence of 0.1 mM L-[ureido-14C]citrulline, 0.5 mM l-aspartate, 20 mM KCl, 0.5 mM MgCl2, 1 mM ATP, and 0.5 μM inorganic pyrophosphatase and in the presence or absence of 22 μM argininosuccinate lyase (ASL). The reaction was halted with PCA (final concentration 2%), and l-arginine was separated from l-citrulline by HPLC (see HPLC). ASS and ASL enzymatic activities were also determined in the rat liver as a positive control. In that case, the liver was homogenized in the same buffer, centrifuged at 14,000 g for 4 min and the supernatant was used for the assay. Ornithine decarboxylase (ODC) activity was measured at 30°C in 100 μl of a HEPES/NaOH buffer (50 mM, pH 7.2) containing 60 μM L-[1-14C]ornithine, 0.5 mM diithiothreitol, 0.2 mM pyridoxal 5'-phosphate, and with or without 10 μM DFMO. ODC activity was calculated from the difference between 14CO2 production in the absence or presence of 10 mM DFMO, which totally inhibits ODC activity (3).

**HPLC.** To measure metabolic fluxes in intact cells and enzymatic activities in cellular homogenates, radioactive products were separated from precursors by HPLC and counted by liquid scintillation. L-Citrulline was separated from L-[U-14C]arginine or from NaH14CO3 using a C18 column (Kromasil, Le Mesnil le Roi, France) after O-phthalaldehyde derivatization as described (5). L-Ornithine was separated from L-[U-14C]arginine using a partisphere C18 column (Whatman, Clifton, NJ), and the gradient was described (6). This latter method was also used for l-arginine separation from L-[ureido-14C]citrulline, for l-citrulline separation from L-[U-14C]ornithine, and for urea separation from L-[guanido-14C]arginine.

**Presentation of results.** The production of radioactive metabolites was calculated by reference to the specific activity of the precursors in the incubation medium. Vmax values were calculated using the Line-weaver-Burk representation. The results were expressed as the means ± SE together with the number of independent experiments performed with different cell preparations (n). The statistical significance of differences between mean values was assessed by the Student’s t-test.

**RESULTS**

**Food intake and body weight.** The difference in food intake between the two groups was more ampe in the very beginning of the experiment than after 7 days. Indeed rats fed isocaloric diets containing 20 (P20) or 58% (P58) casein ingested, respectively, 18 and 17 g of food per day on day 7 (Fig. 1A). Probably due to a higher daily food consumption in the control diet group (P20), the animals in this group had a better daily weight gain within the first 2 days of the experient than the animals from the high-protein diet group (P58), which initially loose weight. Thereafter, the daily weight gain was similar in both groups (Fig. 1B). The mean weights of the animals at the end of the experiments (i.e., after 7 days) were not significantly different (P = 0.1) averaging 232.8 ± 5.6 g for the control diet vs. 223.3 ± 5.0 g for the high-protein diet (n = 18).

**Fecal and intestinal pH and NH4+/NH3 concentrations.** Figure 2 shows the pH in the intestinal contents recovered from animals receiving P20 and P58 diets for 2 and 7 days, respectively. Two days after the consumption of the P58 diet, the pH measured in the proximal colon content (but not in the other anatomical segments) was significantly increased compared with the pH measured in the control group (P20; Fig. 2). After 7 days of consumption of the P58 regimen, however, the pH remained not significantly different than the one measured in the proximal colon content recovered from the animals in the P20 group (Fig. 2).

**Fig. 1.** A: daily total food intake of rats fed a 20% casein diet (P20 control diet) and 58% casein (P58) diet for 7 days. The results are expressed as means ± SE and represent 17 independent experiments. *P < 0.05, **P < 0.001 vs. P20. B: cumulative body wt changes of rats fed the 20 or 58% casein diet. The results are expressed as means ± SE and represent 18 independent experiments. *P < 0.05, **P < 0.001 vs. P20.
P20 group (Fig. 3). Lastly, after 7 days of experimentation, NH$_4^+$/NH$_3$ concentrations were significantly higher in the P58 group in the cecum and in the proximal/median colon luminal contents when compared with the values measured in the P20 group (Fig. 3).

Colonic blood NH$_4^+$/NH$_3$ concentrations. When the NH$_4^+$/NH$_3$ concentration was measured in the blood recovered from the right colonic vein, a rise was registered in the group consuming 58% protein when compared with the group consuming 20% protein (Fig. 4). Indeed, 2 and 4 days after the beginning of the hyperproteic diet consumption, the blood NH$_4^+$/NH$_3$ concentration was approximately threefold higher than in the normoproteic group. However, after 7 days, the blood NH$_4^+$/NH$_3$ concentration was only slightly, but significantly, higher than the concentration measured in venous blood obtained from the P20 animals.

Effect of high-protein diet on L-arginine metabolism in isolated colonocytes. When measuring L-[guanido-14C]arginine conversion to radioactive urea in the isolated colonocytes, this conversion was 1.4-fold ($n = 3$, $P < 0.05$ vs. control) and 2.4-fold ($n = 7$, $P < 0.001$ vs. control) higher in the colonocytes isolated from the P58 group when compared with the conversion measured in the colonocytes isolated from the P20 group after 2 and 7 days, respectively (Table 2). When 1 mM L-[U-14C]arginine was used as a precursor, the production of radioactive L-ornithine measured in the P58 group was also significantly increased when compared with the production measured in the P20 group (Table 2). This increased L-arginine flux through arginase leading to increased urea and L-ornithine production in P58 isolated colonocytes was associated with an increase in arginase catalytic activity detected in colonocyte homogenates (Table 3). Indeed, the maximal velocity of arginase activity ($V_{max}$) was found to be higher in the P58 group than in the P20 control group after 2 and 7 days of the experiment (Table 3).

The arginase activities associated with the cytosolic and particulate fractions were found to be both increased in the P58 group both after 2 and 7 days of the experiment (Fig. 5).

Effect of NH$_4$Cl on the conversion of L-arginine to L-citrulline according to the experimental diets. After 2 days of the experimental diet, NH$_4$Cl (1 mM), when present in the incubation medium, failed to increase the basal radioactive L-citrulline production from 1 mM L-[U-14C]arginine both in colonocytes recovered from control P20 and P58 animal groups (Fig. 6). In contrast, in colonocytes isolated from the

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**Fig. 2.** Rats were fed 20 or 58% casein diets for 2 and 7 days, and pH was measured in the intestinal contents of the cecum, proximal, median, and distal colon. The results are expressed as means ± SE and represent 4 independent experiments. *$P < 0.05$ vs. P20.

**Fig. 3.** Rats were fed 20 or 58% casein diets for 2, 4, and 7 days, and ammonia concentrations were measured in the lumen of the large intestine anatomical segments and in feces. The results are expressed as means ± SE and represent 4 independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. P20.

**Fig. 4.** Rats were fed 20 or 58% casein diets for 2, 4, and 7 days, and ammonia concentrations were measured in the blood recovered from the right colonic vein. The results are expressed as means ± SE and represent 9 independent experiments. *$P < 0.05$, ***$P < 0.001$ vs. P20.

**Fig. 5.** Rats were fed 20 or 58% casein diets for 2 and 7 days, and pH was measured in the intestinal contents of the cecum, proximal, median, and distal colon. The results are expressed as means ± SE and represent 4 independent experiments. *$P < 0.05$ vs. P20.

**Fig. 6.** Rats were fed 20 or 58% casein diets for 2, 4, and 7 days, and ammonia concentrations were measured in the blood recovered from the right colonic vein. The results are expressed as means ± SE and represent 9 independent experiments. *$P < 0.05$, ***$P < 0.001$ vs. P20.
P20 group, 10 mM NH₄Cl increased the basal L-citrulline production in P20 fourfold as in P58 colonocytes; 20 mM NH₄Cl increased this production ninefold, and 50 mM NH₄Cl was even more active. No significant differences in L-citrulline production cellular capacity as a function of NH₄Cl concentration could be measured in P58 colonocytes after a 2 days of diet when compared with increases recorded in the P20 group. After 7 days of the experimental diet, the NH₄Cl-provoked increases in L-citrulline production by control P20 colonocytes were close to the values recorded in this group after 2 days (Fig. 6). However, the NH₄Cl-dependent L-citrulline production was found to be significantly lower in colonocytes isolated from the P58 diet (Fig. 6). The decrease in L-citrulline synthesis capacity in colonocytes isolated from the P58 animals was not associated with any measurable decrease in either the CPSI and OCT catalytic activities (Table 3). Lastly, L-citrulline was apparently not used by isolated colonocytes for L-arginine de novo production in the pathway catalyzed by ASS and ASL, representing thus a metabolic end product. Indeed, the incubation of colonocytes isolated from control P20 and P58 groups after 7 days of experimental diets, in the presence of 1 mM L-[ureido-¹⁴C]citrulline, 1 mM L-aspartate used as a cosubstrate in the reaction catalyzed by ASS, and with or without 5 mM L-valine used to inhibit arginase activity failed to lead to any detectable production of radioactive L-arginine in control P20 colonocytes and in P58 colonocytes (data not shown). Furthermore, ASS activity when measured in the presence of an excess of ASL activity (used to convert all the L-arginino-succinate produced by ASS into L-arginine) was found to be very low in both cases, representing 33.95 ± 12.32 pmol·mg protein·¹·60 min⁻¹ (n = 3) in the control group and 31.69 ± 6.46 pmol·mg protein·¹·60 min⁻¹ (n = 3) in the hyperproteic group. This activity was found to be indeed very small when compared with the activity in the liver homogenate used as the positive control, i.e., 95.6 ± 1.4 nmol·mg protein·¹·60 min⁻¹ (n = 3). Conversion on day 7 of L-citrulline to L-arginine without added ASL was found to be close to the limit of detection, not exceeding 10 pmol·mg protein·¹·60 min⁻¹ in both cases.

Effect of a high-protein diet on polyamine metabolism in isolated colonocytes. L-ornithine derived from L-arginine was little used by the colonocytes for polyamine production in the ODC pathway. Indeed, ODC activity measured in colonocyte

Table 2. Effect of the experimental diets on the L-arginine metabolism in isolated colonocytes

<table>
<thead>
<tr>
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<th>pmol·10⁶·30 min⁻¹</th>
<th>2 days</th>
<th>7 days</th>
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<tr>
<td>Production of urea</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>from 1 mM L-[guanido-¹⁴C]arginine</td>
<td></td>
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<tr>
<td>P20</td>
<td>384.6±67.3</td>
<td>257.9±56.5</td>
<td></td>
</tr>
<tr>
<td>P58</td>
<td>527.7±29.3*</td>
<td>613.1±65.6***</td>
<td></td>
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<tr>
<td>Production of L-ornithine from 1 mM L-[¹⁴C]arginine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P20</td>
<td>419.8±83.7</td>
<td>550.0±170.5</td>
<td></td>
</tr>
<tr>
<td>P58</td>
<td>677.0±111.5**</td>
<td>876.0±261.9*</td>
<td></td>
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</tbody>
</table>

Values are means ± SE of 3–7 independent experiments. Urea and L-ornithine production were measured in colonocytes isolated after 2 or 7 days of the experimental diets. *P < 0.05, **P < 0.01, ***P < 0.001 vs. P20.

Table 3. Effect of the experimental diets on arginase, CPSI and OCT catalytic activities

<table>
<thead>
<tr>
<th></th>
<th>Vmax, nmol·mg protein·¹·60 min⁻¹</th>
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<tbody>
<tr>
<td></td>
<td>Arginase</td>
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<td>2 Days diet</td>
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<tr>
<td>P20</td>
<td>40.9±7.9</td>
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<tr>
<td>P58</td>
<td>100.7±14.9**</td>
</tr>
<tr>
<td>7 Days diet</td>
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<td>P20</td>
<td>45.6±12.5</td>
</tr>
<tr>
<td>P58</td>
<td>102.0±16.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5–8 independent experiments. Enzymatic activities were measured on colonocyte homogenates obtained after two and seven days of the P20 or P58 diet ingestion. *P < 0.05, **P < 0.01 vs. P20. CPSI, carbamoyl phosphate synthetase I; OCT, ornithine carbamoyltransferase.
homogenates recovered from the control P20 and P58 groups averaged, respectively, 2.26 ± 0.57 and 3.75 ± 0.85 pmol·mg protein⁻¹·60 min⁻¹ (n = 4 in both cases) after 2 days of experimental diet and 1.87 ± 0.38 and 3.23 ± 0.85 pmol·mg protein⁻¹·60 min⁻¹ (n = 5 in both cases) after 7 days.

DISCUSSION

Our data clearly confirm that colonic luminal NH₄⁺/NH₃ concentrations increase as a result of enhanced protein intake and demonstrate that this exposure to an increased NH₄⁺/NH₃ environment is associated with modifications of ammonia-related amino acid metabolic capacities in colonicocytes.

As previously described (31), the rats fed the P58 diet are apparently able to adapt to the high-protein diet, because their food intake, although depressed at the beginning of the experiments, was similar to those of the P20 control group from days 3 to 7. A putative brain adaptation to increased plasma NH₄⁺/NH₃ levels (36), together with the adaptation of the liver urea cycle enzymes to a high-protein diet (27) leading to a rapid decrease of plasma NH₄⁺/NH₃ levels, may partly explain the recovery of the normal food intake.

The rats receiving the hyperproteic diet were found to show higher NH₄⁺/NH₃ concentrations in the cecum and in the proximal and median colon lumen after 2 days of the experimental diet and thereafter. NH₄⁺/NH₃ increase was more modest in the distal colon and in the feces. This may result from different bacterial NH₄⁺/NH₃ production and/or utilization for the de novo synthesis of proteins in the different large intestine segments as well as different NH₄⁺/NH₃ absorptive capacities in these segments. Increase of the fecal NH₄⁺/NH₃ concentration can be measured in humans consuming a high-protein diet (12, 14). The dietary protein levels also influenced the NH₄⁺/NH₃ concentration in the blood collected from the colonic vein, which was much higher in the P58 group within the first days of the experiment and then almost returned back to the control values after 7 days, suggesting a metabolic adaptation of colonicocytes toward increased NH₄⁺/NH₃ ammonia concentration.

We previously demonstrated (25) in colonicocytes the metabolic capacity for an incomplete ornithine cycle, acting as a probable detoxification pathway against ammonia. In these cells, the direct conversion of l-arginine into l-citrulline and nitric oxide through nitric oxide synthase activity is negligible when compared with the sequential conversion of l-arginine into l-citrulline through arginase and OCT activities (25, 28). The already described adaptation of the hepatic urea cycle enzymes to an increased protein intake led us to study colonic epithelial cell metabolism in response to increased luminal ammonia concentration. Therefore, we measured the effects of the P58 diet on the metabolic pathways involved in ammonia metabolism in colonicocytes. In our study, we showed that the l-arginine flux through arginase, which allows l-ornithine and urea production, was increased 2 days after the P58 diet ingestion, and this increase was found to be maintained up to the end of the experiments. The increased l-arginine flux through arginase could be explained by a concomitant increase of both cytosolic and particulate arginase activities measured in homogenates of colonicocytes recovered in the hyperproteic group of animals. Studies with increased intake of dietary protein (i.e., 60% casein) indicate that the rat liver shows a marked stimulation in its rate of l-ornithine catabolism (30). Moreover, l-ornithine availability has been shown to limit the rate of urea synthesis in isolated hepatocytes (20). Furthermore, a substantial fall of l-ornithine plasma concentration has been measured under conditions of high portal NH₄⁺/NH₃ concentrations (24). l-Ornithine is not a constituent of proteins, and increasing protein intake thus induces an increased liver l-ornithine requirement for liver ammonia detoxification. The marked increase of arginase activity in colonicocytes by a high-protein diet and the consequent increase in l-ornithine production (without any increase in the l-ornithine requirement for l-citrulline and polyamine synthesis) thus likely contribute to ensure a sufficient l-ornithine production available for its metabolism in the liver. These results suggest that in addition to small intestine enterocytes that release l-ornithine into the blood (4, 41), colonic epithelial cells are involved in a colonic loop axis for an increased l-ornithine supply to the liver in response to a high-protein diet intake. However, it is not possible to exclude in our experiments a role of decreased dietary content in carbohydrates in the isocaloric hyperproteic group of animals for the recorded increase of l-ornithine production capacity by colonicocytes. Indeed, it has recently been reported (2) that a low-carbohydrate/high-fat diet resulted in a slight increase of the postabsorptive plasma l-ornithine concentration when compared with an isocaloric isoproteic high-carbohydrate/low-fat diet.

It is worth noting that increased arginase activity in the colonicocytes leads to increased urea production, which may diffuse in the lumen and serve as a substrate for bacterial ureases leading to increased NH₄⁺/NH₃ production. However, urea represents a minor source of NH₄⁺/NH₃ in the colon (21).

A measurable amount of l-ornithine derived from l-arginine was found to be converted into l-citrulline in the presence of NH₄Cl. l-Citrulline appeared to be the end product of ammonia metabolism in colonicocytes, because these cells did not convert l-citrulline back to l-arginine. This undetectable l-citrulline conversion to l-arginine in colonicocytes can be explained by the very weak ASS activity in homogenates of colonicocytes recovered from normoproteic and hyperproteic groups of animals, indicating that the high-protein diet did not induce ASS activity. A similar metabolic situation is found in the small intestine of weaned animals (41), where l-citrulline produced by enterocytes is recovered in the portal vein, bypasses the liver, and is finally converted into l-arginine by the kidney, allowing, through this intestine-kidney axis, the de novo synthesis of l-arginine by the body (42).

After 2 days of the experimental diet, l-citrulline production by the colonicocytes in the presence of NH₄Cl was not significantly modified in the hyperproteic group when compared with the control group. In contrast, a marked significant decrease of colonicocyte capacity to synthesize l-citrulline was evidenced in the hyperproteic P58 group after 7 days of the experimental diet. Surprisingly, this decreased metabolic flux did not correspond to any decrease in CPSI and OCT activities measured in homogenates of colonicocytes recovered from the hyperproteic group. NH₄Cl concentrations used in the incubation medium to increase l-citrulline synthesis (i.e., up to 50 mM) were in the range of the NH₄⁺/NH₃ concentrations found in the colon lumen, concentrations that have been shown to exert no short-term cytotoxic effect on isolated colonicocytes (25). In the hyperproteic group, the decrease in the colonicocyte capacity for
L-citrulline production from L-arginine in the presence of NH₄Cl could thus not be explained by a decrease of the colonocyte capacity to generate L-ornithine from L-arginine, by an increased utilization of L-ornithine for polyamine synthesis, or by a decrease of the colonocyte capacity to metabolize ammonia. The most likely explanation is that the L-citrulline production capacity in colonocytes is limited by the ammonia availability for cellular metabolism.

NH₄+/NH₃ generated in the luminal contents has been suggested to increase luminal pH (19). However, in the present study, significant pH differences associated with different levels of protein intake were found only in the luminal content of the proximal colon recovered after 2 days of experimental diet from the hyperproteic group of animals. Although it is obvious that luminal pH can be modified in vivo by numerous luminal compounds other than NH₄+/NH₃, an increase in luminal pH may favor ammonia entry into the colonic cells. Indeed, because ammonia is a weak base with a pKₐ equal to 9.02, the vast majority of ammonia produced by the bacterial flora exists as NH₄+/NH₃ in the colonic luminal environment (40). Thus the higher protein consumption and the associated higher luminal pH in the proximal colon after 2 days of the experimental diet will result in an enhanced ratio of nonionized NH₃ over the protonated NH₄+ form and the absorption of NH₃ by nonionic diffusion. Indeed, NH₃ passage through cellular membranes is 400 times more rapid than for NH₄+ (11). In other words, the in vivo transient increase of luminal pH may participate in the rapid increased ammonia movements through the surface epithelial colon cells. In colonic crypt cells, a strong permeability barrier has been shown to limit NH₃ diffusion (37). However, in apical membranes from rat distal colon, the H⁺/K⁺-ATPase has been shown to be able to substitute NH₄+ for K⁺, raising the view that this enzyme may function as an NH₄+ pump (10). In our study, the colonocyte isolation procedure used leads to the recovery of surface epithelial absorbing colonocytes, which represent ~90% of the isolated cells (9), the remainder being goblet cells.

Altogether, our results (see Fig. 7) show that in response to increased NH₄+/NH₃ concentration in the colonic luminal content, colonocytes are able to increase L-ornithine synthesis from L-arginine and to decrease L-ornithine utilization for L-citrulline synthesis. The resulting higher L-ornithine availability would correspond to an elevated requirement for the elimination of portal blood ammonia in the liver urea cycle. It is conceivable that in a situation of plethoric alimentary protein supply, the intestinal production of L-citrulline used for kidney L-arginine de novo synthesis would be lowered. From the NH₄+/NH₃ concentrations measured in the large intestine lumen and venous blood at different time points following hyperproteic diet ingestion, it appears that the increased NH₄+/NH₃ movement through the colonic epithelium is transient and limited. The adaptive metabolic capacities of the differentiated absorbing colonocytes toward NH₄+/NH₃ likely contribute to regulate both intracellular NH₄+/NH₃ concentration and ammonemia. Further work is required to get a better understanding of the metabolic events involved in the adaptation of the colonic epithelial cells to increased luminal ammonia concentrations, particularly after longer periods of hyperproteic diet consumption. This is of considerable interest, because excessive luminal NH₄+/NH₃ concentrations are considered to be deleterious for colonic epithelium (18, 39) and because colonic epithelium can be viewed in an interorgan metabolic perspective as a first line of defense against luminal NH₄+/NH₃ entry into the bloodstream.

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**Fig. 7.** Schematic view for the evolution of the metabolic capacities of colonic epithelial cells after 7 days of hyperproteic (P58) vs. normoproteic (P20) diet ingestion. ARG, arginine; CITR, citrulline; CP, carbamoylphosphate; CPSI, carbamoylphosphate synthetase I; OCT, ornithine carbamoyltransferase; ODC, ornithine decarboxylase; ORN, ornithine; P, putrescine.
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