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The effect of alimentary polyamine depletion on germ-free and conventional rats

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Polyamine-deficient semisynthetic diet or polyamine-rich standard rat chow (Altromin 1320) were fed to germ-free and conventional rats to study the influence of alimentary polyamine intake on the endogenous polyamine content and the polyamine formation by the intestinal microflora.

Putrescine was the major polyamine in the intestinal contents or feces of germ-free rats. In contrast, the intestinal contents and feces of conventional rats contained mainly spermidine, but only low concentrations of putrescine and spermine. Cadaverine was not detected at all. These polyamine patterns were not affected by the dietary polyamine intake. The polyamine patterns of blood plasma and colonic tissue were similar in germ-free and conventional rats: putrescine was the major polyamine in plasma, whereas spermidine and spermine predominated in colonic tissue. The concentrations of putrescine in plasma and of spermidine and spermine in colonic tissue were lower in rats fed the polyamine-deficient semisynthetic diet than in rats fed the Altromin diet. This difference was greater in germ-free than in conventional rats. Bacteroides, Eubacterium, *and* Lactobacillus *were the predominant organisms of the fecal flora found in conventional rats. The composition of the microflora differed only slightly in response to the diet.*

In conclusion, (1) putrescine is the main endogenously generated polyamine secreted into the gut lumen, (2) the high spermidine content in the luminal content of all intestinal segments of the conventional rats was independent of the diet and therefore must be of microbial origin, and (3) the intraluminal microbial polyamine formation seems to be inversely related to the alimentary polyamine supply. © Elsevier Science Inc. 1996 (J. 560–566, 1996.)

Keywords: Diet; polyamine; germ-free rat; conventional rat; intestinal microflora

Introduction

The polyamines putrescine, spermidine, and spermine are polycationic compounds which are synthesized by both eukaryotic and prokaryotic cells.[1] Due to their specific interaction with nucleic acids,[2,3] polyamines have been implicated in a variety of cellular processes such as replication and transcription as well as cell growth and differentiation.[4–6] It is well accepted that polyamines play an important role in adaptive growth of the intestinal mucosa after injury or surgery[7–10] and in its maturation.[11–13] Furthermore, polyamines are important factors in tumor growth.[14]

The first step in polyamine biosynthesis is catalyzed intracellularly by the enzyme ornithine decarboxylase (ODC; E.C. 4.1.1.17). DL, α-difluoromethyl-ornithine (DFMO) is a widely used ODC inhibitor. The cytostatic effect of DFMO was demonstrated in a variety of cell cultures and in selectively or totally decontaminated mice.[15,16] However, administration of DFMO to conventional animals or patients with tumors had insufficient cytostatic effects.[16,17] This phenomenon could be explained by the availability of polyamines in the intestinal lumen. Possible sources of intestinal polyamines are the polyamines released during the normal body cell turnover, dietary polyamines, and those produced by the intestinal microflora.[18–21] The importance of microbially formed polyamines is supported by the observation that in conventional rats the arginine decarboxylase, which catalyzes the formation of putrescine in prokaryotes, has a considerably higher activity in the intestinal content than in the mucosa.[22] Further support for this notion

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comes from the observation that the urinary excretion of cadaverine, which is formed exclusively by bacteria, decreased during total antibiotic elimination of bacteria in healthy persons and increased after reestablishment of bacteria in the digestive tract.[23] Feeding experiments with conventional and germ-free animals provide an opportunity to distinguish endogenous polyamines from those of microbial origin. The objective of our present study was to gather basic information on the intraluminal polyamine concentrations at various locations in the intestinal tract, in blood plasma, and in colonic tissue of germ-free and conventional rats fed diets with different polyamine contents. The results obtained from the two animal models should extend our knowledge about the influence of dietary polyamines on the endogenous polyamine content and on the intraluminal polyamine formation by the intestinal microflora. The study of the composition of the intestinal microflora in conventional rats should provide information on those bacterial population groups in rats that are responsible for polyamine production.

Methods and Materials

Chemicals

Usual laboratory chemicals were obtained from Merck (Darmstadt, GFR) or Fluka Chemie (Neu-Ulm, GFR). Polyamine standard substances were purchased from Sigma-Aldrich Chemie GmbH (Deisenhofen, GFR).

Diets

Polyamine-rich diet. Standard rat chow Altromin 1320 from Altromin GmbH (Lage, GFR).

Polyamine-deficient semisynthetic diet. The following components were thoroughly mixed: 23g casein corresponding to 20g protein (Dauermilchwerk Peiting GmbH Landshut, GFR), 65g wheat starch (Heller & Strauss, Berlin, GFR), 5g sunflower oil (Thomy GmbH, Karlsruhe, GFR), 2g microcrystalline cellulose (Rettenmeier & Söhne GmbH, Ellwangen, GFR), 5g mineral mix (Altromin GmbH, Lage, GFR), and 3g 'vitamin mix, standard diet' for conventional rats or 3g 'vitamin mix, standard diet fortified' in the case of germ-free rats (Altromin GmbH, Lage, GFR). The gross energy content of the diet was 1654.8 kJ per 100g dry matter. Portions of 25g (dry matter) of the polyamine-deficient diet were sealed in polyethylene bags and sterilized by radiation.

Experimental design

Animals. Two groups of conventional and germ-free male Wistar rats (Central Institute of Experimental Animal Sciences, Hannover) weighing 191 ± 31g were used. Rats were individually housed in wire-bottomed cages with a 12-hr light:dark cycle and a room temperature of 22 ± 2°C. The cages of the germ-free animals were arranged in germ-free isolators equipped with a sterile water supply. Material from each isolator was checked weekly for sterility. One group each of the conventional (8 animals) and of the germ-free rats (10 animals) received Altromin 1320 (standard rat chow). The other two groups (conventional rats, $n = 6$, germ-free rats, $n = 10$) were fed the semisynthetic polyamine-deficient diet. Animals were adapted to the polyamine-deficient diet for 7 days. The experimental period was 6 days for all four feeding groups. Because there were no differences in food consumption and weight gain between the different feeding groups during the adaption period, the diets and water were provided ad libitum. During the study, feces were collected from each animal and food intake was measured daily. Each animal was weighed at the beginning and at the end of the study. The protocol was approved by the Animal Welfare Committee of Brandenburg.

Sample collection and preparation

On day 7 of the experimental period, rats were individually sacrificed by ether anesthesia and dissection of the *vena jugularis* at 8:00 a.m. Blood was collected in EDTA-pretreated vials and centrifuged immediately at 980 g for 5 min at 4°C to separate blood plasma and blood cells. The extraction of polyamines was done by adding 0.1 mL 50% (w/v) TCA to 1 mL plasma and subsequent centrifugation at 980 g for 5 min at 4°C. Aliquots of the supernatant were used for polyamine analysis. Luminal contents were removed from the cecum and colon. In addition, tissue samples were collected from the colon and promptly rinsed with ice-cold isotonic NaCl. All samples were frozen in liquid nitrogen and subsequently stored at −85°C. Previous studies had indicated a complete recovery of polyamines after lyophilization. Therefore, prior to analysis, the intestinal content and tissues were lyophilized and stored at 4°C in a desiccator. The extraction of the polyamines from the intestinal samples was performed by homogenization of approximately 0.2 g of the lyophilized sample in 1.5 mL 5% (w/v) TCA and centrifugation at 26,000 g for 30 min at 5°C. The supernatant was collected and the sediment was washed with 1.5 mL 5% (w/v) TCA and recentrifuged. Before analysis, aliquots of the combined supernatants were centrifuged through Ultrafree MC-filter units with a pore size of 0.2 μm (Millipore). The diets were subjected to the same extraction procedure used for the intestinal contents, whereas the colonic tissues (approximately 0.2 g) were placed in an ice bath, homogenized in 1.5 mL 5% (w/v) TCA, sonicated three times for 30 sec, and centrifuged as described. For the microbiological studies, approximately 0.5 g of feces was immediately placed into preweighed tubes with 2.0 mL of a prereduced brain-heart infusion broth (DIFCO). The analyses of short-chain fatty acids (SFCA) were performed with aliquots of freshly collected feces. Samples were prepared as described by Pomare et al.[24] Samples of 1 μL were injected into a Hewlett Packard 5890, Serie II gas liquid chromatograph fitted with a 25-m Carbowax 20 M capillary column (inside diameter 0.32 mm; Hewlett Packard, USA) and a flame ionization detector. The column was held at 125°C with helium as the carrier gas. The flow rate was 12 mL/min. The splitting rate was set at 1:10. All samples were analyzed in duplicate.

Polyamine analysis

The polyamine contents of the intestinal lumen, tissue, and plasma were determined by HPLC (GYNKOTEK, Germering, Germany). The polyamine assay was optimized in our laboratory (paper in preparation). The polyamines were separated on a cation-exchange column and quantified after postcolumn derivatization with ninhydrin. With this method, amino acids and small peptides were eluted with the buffer front, whereas all polyamines were well separated from each other. The retention times for putrescine, cadaverine, spermidine, and spermine were 18.6 min, 21.8 min, 33.0 min, and 56.3 min, respectively. The detection limit of each polyamine was 20- to 35 pmol/20 μL injection volume. The standard deviation of the assay was ± 1.3%.

Dry weights

Gut contents and colonic tissues were freeze-dried to constant weight under vacuum.

Microbial studies

Following homogenization, the specimens were subjected to a series of 10 fold dilutions (10^{-2} to 10^{-8}) in a prereduced saline buffer[25] and duplicate samples of 0.05 mL of each dilution were plated on non-selective and selective media.[26] All manipulations of the anaerobic media were performed in an anaerobic chamber (MK3 anaerobic workstation, dW Scientific, England). The inoculated media were incubated at 37°C for 4 days (anaerobic bacteria) or for 1 to 2 days (aerobic microorganisms). Total colony counts were determined from the anaerobic and aerobic blood agar plates. The criteria used for identification of anaerobic isolates were those outlined by the Anaerobic Laboratory at Virginia Polytechnic Institute and State University.[27] The viable counts are expressed as $\log_{10}$ of colony-forming units (cfu)/g dry weight of feces.

Statistical procedure

Data are expressed as means ± SD. Student's *t*-test was used in the statistical evaluation, and values of $P < 0.05$ were regarded as significant.

Results

Polyamine intake

The polyamine intake of germ-free and conventional rats fed either Altromin 1320 or semisynthetic polyamine-deficient diet was calculated from the polyamine contents of the various diets and the daily food intake. The concentrations of putrescine, spermidine, and spermine were 3.3 ± 0.2, 46.1 ± 2.8, and 18.1 ± 1.4 nmol/g dry weight for the polyamine-deficient diet, and 279 ± 14, 219 ± 15, and 213 ± 15 nmol/g dry weight for Altromin, respectively. These values refer to the free acid soluble polyamines in the diets. Feeding the polyamine-deficient diet reduced the dietary intake of putrescine, spermidine, and spermine to 1.2%, 21.1%, and 8.5%, respectively, as compared with the Altromin diet. Feeding the various diets did not result in any differences in the daily food intake or in weight gain in both germ-free and conventional rats (*Table 1*).

Table 1 Intake of food and polyamines and weight gain of germ-free and conventional rats fed either Altromin or polyamine-deficient diet

	Germ-free rat	Conventional rat
Food intake [g dry weight/animal × d]		
Altromin 1320	22.0 ± 2.0	23.2 ± 1.8
Polyamine-deficient diet	19.6 ± 1.9	21.5 ± 2.1
Polyamine intake [μmol/animal × d]		
Altromin 1320:		
Putrescine	6.14 ± 0.56	6.47 ± 0.52
Spermidine	4.82 ± 0.44	5.06 ± 0.40
Spermine	4.69 ± 0.43	4.94 ± 0.40
Polyamine-deficient diet:		
Putrescine	0.065 ± 0.01	0.071 ± 0.01
Spermidine	0.904 ± 0.08	0.991 ± 0.10
Spermine	0.355 ± 0.03	0.389 ± 0.04
Weight gain [g/animal × d]		
Altromin 1320	3.8 ± 1.5	4.5 ± 1.6
Polyamine-deficient diet	2.9 ± 1.3	3.7 ± 0.9

Rats were adapted to the polyamine-deficient diet for 7 days. The experimental period was 6 days for all feeding groups as described under Experimental design. Values are means ± S.D. of germ-free (10 rats for each group) and conventional rats (eight rats were fed Altromin 1320 and six rats, the polyamine-deficient semisynthetic diet, respectively).

The polyamine contents of the intestinal lumen and feces in germ-free and in conventional rats

Independent of the diet, putrescine was the predominant polyamine in the luminal content of cecum and colon and in feces of germ-free rats (*Table 2*). Cadaverine was not detected. Moreover, there were no differences in polyamine concentrations between the luminal contents of germ-free rats fed Altromin or the polyamine-deficient diet.

Conventional rats differed considerably from germ-free rats in that they contained spermidine instead of putrescine as the major polyamine in intestinal and fecal samples (*Table 3*); this was independent of the diet. Cadaverine was not detected in any case. The polyamine-deficient semisynthetic diet fed to conventional rats resulted in significantly higher intraluminal spermidine concentrations in cecum and colon than the Altromin diet.

Plasma and colonic tissue polyamine concentrations

Putrescine was the predominant polyamine in plasma of both germ-free and conventional rats irrespective of whether Altromin or polyamine-deficient diet was fed (*Figure 1*). However, significantly higher concentrations of plasma putrescine were observed in germ-free and in conventional rats fed on Altromin (127.5 ± 12.9 and 88.5 ± 11.6 nmol/mL, respectively) than in rats fed on polyamine-deficient diet (59.9 ± 13.8 and 52.5 ± 16.8 nmol putrescine/mL in germ-free and conventional rats, respectively). Thus, the polyamine-deficient diet caused a decrease in plasma putrescine by 53% in germ-free or 40.7% in conventional rats in comparison with the Altromin diet.

The polyamine contents of colonic tissue were studied in germ-free and conventional rats in response to both types of diet. It is evident from *Figure 2* that the relative concentrations of the individual polyamines in the colonic tissue were

Table 2 Polyamine concentrations (μmol/g dry weight) in the luminal content of various gut segments and in feces of germ-free rats fed Altromin ($n = 10$) or polyamine-deficient diet ($n = 10$)

	Altromin 1320	Polyamine-deficient diet
Cecum:		
Putrescine	4.19 ± 0.79	3.15 ± 0.85
Spermidine	0.41 ± 0.14	0.48 ± 0.21
Spermine	0.35 ± 0.04	0.40 ± 0.10
Colon:		
Putrescine	3.89 ± 0.65	3.42 ± 0.49
Spermidine	0.49 ± 0.21	0.54 ± 0.16
Spermine	0.42 ± 0.09	0.44 ± 0.14
Feces:		
Putrescine	2.09 ± 0.15	2.04 ± 0.50
Spermidine	0.48 ± 0.08	0.21 ± 0.11
Spermine	0.41 ± 0.07	0.33 ± 0.09

Values are means ± S.D.

Table 3 Polyamine concentrations (μmol/g dry weight) in the luminal content of various gut segments and in feces of conventional rats fed Altromin (n = 8) or polyamine-deficient diet (n = 6)

	Altromin 1320	Polyamine-deficient diet
Cecum:		
Putrescine	<0.005	0.20 ± 0.08
Spermidine	2.94 ± 0.79	4.38 ± 0.92*
Spermine	0.27 ± 0.07	0.63 ± 0.17
Colon:		
Putrescine	<0.005	0.16 ± 0.12
Spermidine	2.25 ± 0.54	3.23 ± 0.85*
Spermine	0.18 ± 0.05	0.47 ± 0.10
Feces:		
Putrescine	0.21 ± 0.10	0.13 ± 0.07
Spermidine	1.23 ± 0.26	1.74 ± 0.60
Spermine	0.11 ± 0.05	0.26 ± 0.06

Values are means ± S.D., significant differences, Altromin 1320 vs. polyamine-deficient diet: *$P < 0.02$ (Student's *t*-test).

similar in both germ-free and conventional rats, irrespective of the diet fed. In both groups of animals, spermidine and spermine were present in high concentrations, whereas putrescine was found in low concentrations or not detected at all. As previously described for the polyamine contents in plasma, the polyamine-deficient diet affected the polyamine contents of the colonic tissue in both groups of rats in a similar way. The concentrations of spermidine and spermine were generally lower with the polyamine-deficient diet (spermidine 1.80 ± 0.89 versus 1.55 ± 0.24; spermine 1.83 ± 0.3 versus 1.32 ± 0.1 μmol/g dry weight; germ-free versus conventional rats, respectively) than with the Altromin diet (spermidine 3.15 ± 0.26 versus 2.14 ± 0.74 μmol/g dry weight; spermine 3.55 ± 0.29 versus 2.59 ± 0.30 μmol/g dry weight, respectively).

Short-chain fatty acid content in feces

The short-chain fatty acids (SCFA) were determined in freshly collected feces of germ-free and conventional rats fed either Altromin or the polyamine-deficient diet to characterize the intestinal environment under the feeding conditions used and the microbial fermentation activity in the gut lumen of conventional rats. In germ-free rats, acetic acid was the only SCFA detected (*Table 4*). The concentration of acetate amounted to 81.4 ± 14.4 μmol/g dry weight in Altromin-fed rats and 70.5 ± 9.8 μmol/g dry weight in rats fed the polyamine-deficient diet. The conventional rats responded to the two diets with differences in the fecal concentrations of SCFA. Whereas the total fecal concentration of SCFA in Altromin-fed rats was 146.9 ± 44.1 μmol/g dry weight, the rats on the polyamine-deficient diet had only 20.2 ± 4.4 μmol/g dry weight in the feces. Acetate, propionate, butyrate, n-valerate, and i-valerate were the principle SCFA observed. The molar fecal SCFA ratios were 56.6% acetate, 13.5% propionate, 26.9% butyrate, and 1.5% n- or i-valerate for the Altromin-fed rats and 74.3% acetate, 17.8% propionate, and 7.9% butyrate for the rats on the polyamine-deficient diet. Of the latter feeding group, n-valeric and i-valeric acid were not detected in the feces.

The fecal flora composition of conventional rats

The composition of the fecal flora was studied in conventional rats fed Altromin or polyamine-deficient diet (*Table 5*). This was done to find out whether the different diets might result in the stimulation of a particular intestinal population group, which in turn, might be responsible for the differences observed with respect to the SCFA and polyamines.

There were few differences in bacterial counts between the two feeding groups. Bacteroides, eubacteria, and lactobacilli were the numerically predominant organisms of the fecal flora in both feeding groups. The counts of facultatively anaerobic lactobacilli and streptococci tended to be lower ($P < 0.05$) in the feces of rats fed the polyamine-deficient diet than of rats fed the Altromin diet. Bifidobacteria were not detected.

Discussion

Feeding trials with germ-free and conventional rats were performed to investigate the influence of the alimentary polyamine intake on the endogenous polyamine levels and the impact of the intestinal microflora on the host's polyamine status. In our study, high putrescine concentrations combined with low spermidine and spermine concentrations were observed in the luminal content from all the various intestinal locations and in the feces of germ-free rats. Cadaverine was never detected. In contrast, the feces of completely decontaminated mice fed a purified diet contain extremely low but equal concentrations of putrescine, spermidine, and spermine.[16] Cadaverine, which is produced exclusively by bacteria, was detected as well. These results indicate that germ-free animals and totally decontaminated conventional animals differ with respect to their polyamine metabolism.

In our study, the dietary polyamine intake by germ-free rats was not reflected by the intraluminal polyamine concentrations of the animals. This may be due to a rapid absorption or degradation of alimentary polyamines in the small intestine.[28–33] Therefore, the intraluminal polyamine contents of the germ-free rats may reflect the relative concentrations of the endogenous polyamines. The similarity of the polyamine patterns of plasma and intestinal content observed in this study are in accordance with this notion. These findings may be explained by pancreaticobiliary secretions of polyamines into the duodenum.[34]

In conventional rats, the polyamine patterns of intestinal content and feces are characterized by high contents of spermidine and low levels of putrescine and spermine, independent of diet.

The results obtained with germ-free rats in our study indicated that (1) dietary polyamines were rapidly absorbed by the mucosal cells of the small intestine and (2) putrescine was the main endogenous polyamine secreted into the gut lumen. The high concentration of spermidine in the lumen of all intestinal segments of the conventional rats, therefore, may not be attributed to the dietary intake or the endogenous secretion of polyamines, but suggests a role of the intestinal microflora in the formation of spermidine. Furthermore, intraluminal pattern in conventional rats may

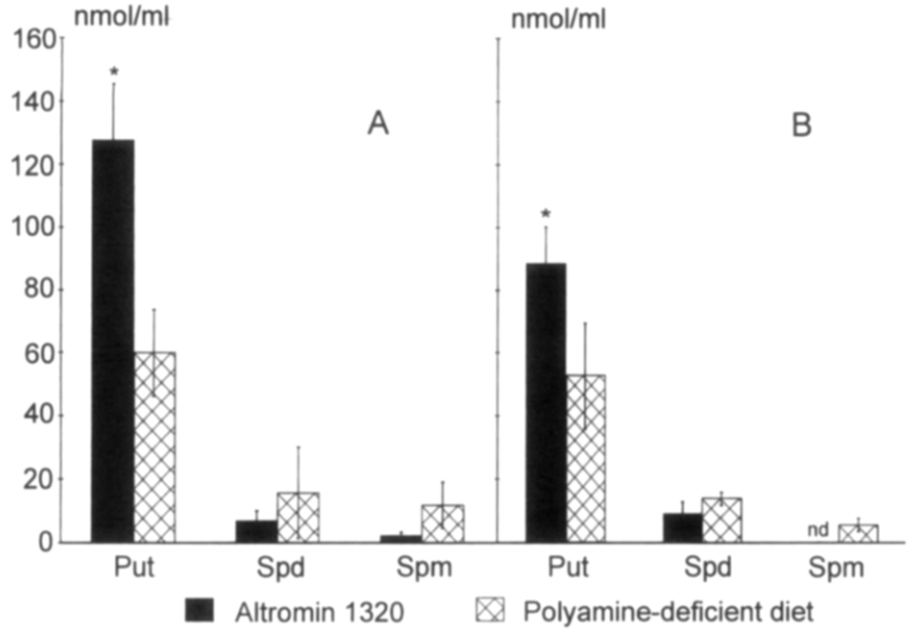

Figure 1 Plasma polyamine concentrations (means ± S.D.). Panel A: germ-free rats fed Altromin 1320 or polyamine-deficient semisynthetic diet (10 rats for each group). Panel B: conventional rats fed Altromin 1320 (eight rats) or polyamine-deficient semisynthetic diet (six rats). Rats were adapted to the polyamine-deficient diet for 7 days. The experimental period was 6 days for all feeding groups as described under *Experimental design*. Significant differences, Altromin 1320 vs. polyamine-deficient diet: $^{*}P < 0.001$ (Student's t-test). nd, not detected.

change dramatically in response to certain soluble undigestible polysaccharides (unpublished results). This demonstrates the significance of the intestinal microflora in the animal's polyamine metabolism.

Spermine synthase is not a widespread enzyme in bacteria;[35] in agreement with this finding, we did not observe significant differences in the intraluminal spermine concentrations between germ-free and conventional rats.

Whereas the intraluminal polyamine patterns in germ-free and conventional rats clearly indicate differences with respect to the polyamine sources, the polyamine patterns of plasma or colonic tissue were similar in both animal models. The polyamine composition in the colonic tissue observed in our study is in good agreement with those of previous studies in which various tissues were investigated.[21,36] To investigate the question, whether a small dietary intake of

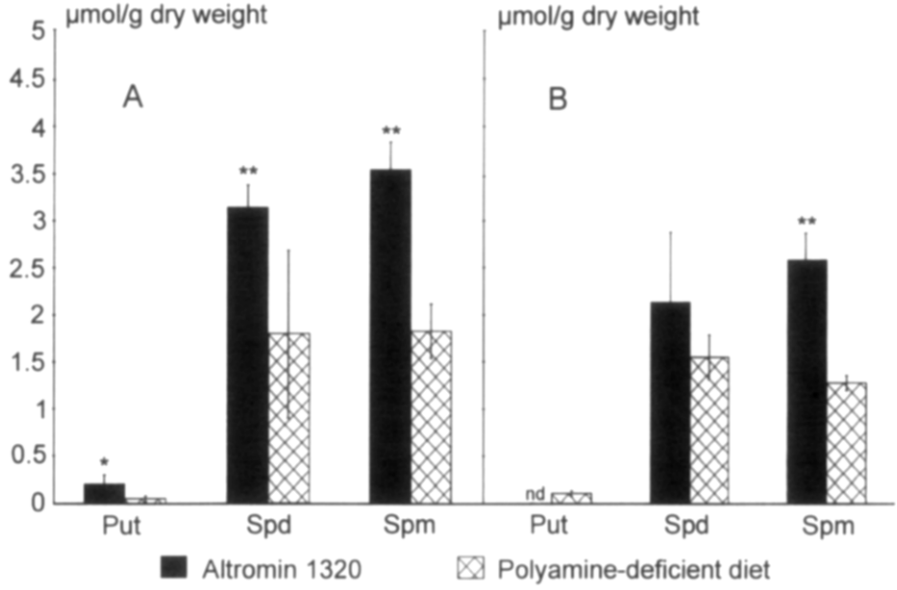

Figure 2 Polyamine concentrations in colonic tissue (means ± S.D.). Panel A: germ-free rats after feeding Altromin 1320 or polyamine-deficient semisynthetic diet (10 rats for each group). Panel B: conventional rats fed Altromin 1320 (eight rats) or polyamine-deficient semisynthetic diet (six rats). Rats were adapted to the polyamine-deficient diet for 7 days. The experimental period was 6 days for all feeding groups as described under *Experimental design*. Significant differences, Altromin 1320 vs. polyamine-deficient diet: $^{*}P < 0.01$, $^{**}P < 0.001$ (Student's *t*-test). nd, not detected.

Table 4 Short-chain fatty acid (SCFA) concentrations [μmol/g dry weight] in feces of germ-free and conventional rats fed Altromin 1320 or polyamine-deficient diet

SCFA	Altromin 1320		Polyamine-deficient diet	
	Germ-free rats	Conventional rats	Germ-free rats	Conventional rats
Acetate	81.4 ± 14.4	83.1 ± 22.6	70.5 ± 9.8	15.0 ± 4.7
Propionate	n.d.	19.9 ± 5.0	n.d.	3.6 ± 1.9
n-Butyrate	n.d.	39.4 ± 17.1	n.d.	1.6 ± 0.8
n-Valerate	n.d.	2.2 ± 0.7	n.d.	n.d.
i-Valerate	n.d.	2.3 ± 0.6	n.d.	n.d.
Total SCFA	81.4 ± 14.4	146.9 ± 44.1	70.6 ± 9.8	20.2 ± 4.4

Values are means ± S.D. of germ-free (10 rats for each group) and conventional rats (eight rats were fed Altromin 1320 and six rats, the polyamine-deficient semisynthetic diet, respectively). n.d., not detected.

polyamines causes accumulation over time in the gut tissue, we calculated the possible uptake of polyamines by the digestive tract of germ-free rats over the entire experimental time of 13 days (7 days adaption to the diet; 6 days experimental period) and compared it with the amounts found in the colonic tissue. The amount of polyamines taken up during this period was calculated on the basis of (1) data obtained by Bardocz et al.[20,33] who found that (a) 8% of [^{14}C]-spermidine applied by intraperitoneal injection was recovered 1 hr after injection in the digestive tract and (b) the recovery of orally applied [^{14}C]-polyamines identified as putrescine, spermidine, and spermine in the jejunal tissue was 11 to 15%, 79 to 82% or 72 to 74%, respectively; (2) the dry weight of the digestive tract of rats (191 g body weight) amounted to 2.1 ± 0.2 g (results of our study). The calculated uptake of putrescine, spermidine, and spermine by the intestinal tract of rats fed the polyamine-deficient diet over 13 days were 3.5 to 4.8, 349 to 361, and 125 to 128 nmol/g dry weight, whereas the contents of these polyamines in the colonic tissue were 50 ± 30, 1800 ± 890, and 1830 ± 290 nmol/g dry weight, respectively. Although our calculation is a very rough estimate, the small calculated uptake of the various polyamines in comparison with the amounts detected in the colonic tissue does not support a considerable accumulation of dietary polyamines in the gut tissue over time.

Table 5 Means ± SD of microbial counts (lg CFU/g dry weight) and frequency of microbial groups (in parenthesis) in the fecal flora of conventional rats fed Altromin 1320 (n = 24) or polyamine-deficient diet (n = 6)

Fecal bacteriology	lg CFU/g dry weight	
	Altromin 1320	Polyamine-deficient diet
Total counts	10.4 ± 0.2	10.0 ± 0.1
Eubacteria	9.3 ± 0.4 (88%)	9.1 ± 0.3 (100%)
Grampos. anaerobic cocci	8.4 ± 0.3 (79%)	8.3 ± 0.3 (100%)
Clostridia	5.9 ± 0.4 (100%)	5.4 ± 0.3 (100%)
Bacteroides/ Fusobacteria	10.1 ± 0.2 (100%)	9.9 ± 0.1 (100%)
Lactobacilli	9.9 ± 0.3 (100%)*	9.2 ± 0.3 (100%)
Streptococci	6.4 ± 0.3 (100%)*	7.0 ± 0.3 (100%)
Staphylococci	4.9 ± 0.4 (100%)	4.4 ± 0.5 (100%)
Enterobacteria	7.4 ± 0.5 (100%)	7.6 ± 0.5 (100%)

Significant differences, Altromin 1320 vs. polyamine-deficient diet: *$P < 0.05$, (Student's t-test).

It is interesting to note that intestinal microflora has been suspected as a source of tissue polyamines, as indicated by the appearance of cadaverine in urine.[16] The similarity in the polyamine contents of colonic tissue of germ-free and conventional rats observed in our study suggests the contribution of the intestinal bacteria to the tissue polyamine levels is negligible. However, in contrast to the intestinal contents, colonic tissue of germ-free and conventional rats fed the polyamine-deficient diet contained lower polyamine concentrations than colonic tissue of rats fed the Altromin diet. The diet-induced changes in the plasma levels of putrescine in both rat models are in accordance with these results. These diet-dependent differences were more pronounced in germ-free than in conventional rats suggesting that intestinal microflora contributes to the polyamine supply of the intestinal mucosa. Furthermore, we can conclude from these results that the microbial contribution to the tissue polyamine levels is probably low if a polyamine-rich diet is available. Possibly, there is an inverse relationship between the polyamine intake and the intraluminal microbial formation of polyamines.

Our data on the composition of the fecal flora indicate that the numerically predominant organisms are non spore-forming strict and facultative anaerobes belonging to the genera *Bacteroides, Eubacterium,* and *Lactobacillus.* Asaccharolytic species of *Bacteroides, Eubacterium,* and *Clostridium* are known to play an important role in the utilization of peptides and amino acids.[37–39] It is probable that the large and diverse population of the intestinal flora is able to adapt its metabolic activities to changes in substrate supply or environmental conditions. Our results are in accord with this assumption. Although the composition of the fecal flora in conventional rats fed either the Altromin or the polyamine-deficient diet differed only slightly, significantly lower amounts of fecal short-chain fatty acids were found in rats fed the polyamine-deficient diet, suggesting that the bacterial enzyme activities were mainly influenced by the intraluminal supply of fermentable substrates from the diet.

The production of polyamines is considered to be a mechanism by which the bacteria can adjust its environmental pH. This is accomplished by the induction of the biodegradative ornithine, arginine, and lysine decarboxylases, whose activities lead to an alkalinization.[40–42]

Which organisms in the intestinal microflora of the rat contribute to the observed polyamine formation and which factors influence this process remain to be determined.

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