The Effect of Oral-administered Lactulose on Colonic Nitrogen Metabolism and Excretion

PER BRØBECH MORTENSEN

Department of Medicine A, Rigshospitalet, University of Copenhagen, DK-2100 Copenhagen, Denmark

The influence of lactulose on organic acid fermentation, nitrogen metabolism and excretion in the colon associated with its mechanism of action on hepatic encephalopathy was investigated. Orally administered lactulose in increasing amounts (0 to 20 to 40 to 80 to 160 gm/day) to 12 healthy volunteers decreased ammonia production in 16.6% fecal homogenates incubated 6 hr and 24 hr at 37° C (mean ± S.E.M.: from 7 ± 1 to 0 ± 0 and from 13 ± 2 to 0 ± 0 mmol/L, respectively). Every dose of lactulose was given for 3 days with intervals of 1 to 2 wk, and 24-hr stools were collected on day 3. Fecal concentrations of ammonia decreased (from 50 ± 9 to 11 ± 3 mmol/L), but ammonia excretions increased (from 6 ± 2 to 17 ± 4 mmol/24 hr). Total fecal concentrations of nitrogen decreased (from 1,043 ± 78 to 300 ± 136 mmol/L), but excretions of nitrogen increased fourfold (from 111 ± 21 to 457 ± 113 mmoL/24 hr) because of the increase in stool mass. Fecal pH declined (from 6.9 ± 0.1 to 4.9 ± 0.1), but total organic acids (short-chain fatty acids and DL-lactate; range = 105 to 148 mmol/L) and osmolality in feces (417 to 450 mOsm/L) did not change, although the colonic fermentation of lactulose had a major impact on the proportions between the nontoxic acetate (increased from 65% ± 2% to 89% ± 3%) and the potentially neurotoxic 3-6-carbon fatty acids (decreased from 35% ± 2% to 11% ± 2%). The effects of lactulose on bacterial ammonia assimilation, protein degradation and fermentation in the colonic contents *in vivo* are central in the mechanism of action for the increased excretion of nitrogen. However, ammonia excretion *per se* is not important because it only constitutes 4% to 5% of fecal nitrogen. (HEPATOLOGY 1992;16:21350-1356.)

When lactulose was first used to treat hepatic encephalopathy approximately 25 yr ago, it was assumed that its beneficial effects were mediated by way of changes in the enteric flora (1, 2). More recently, it has become apparent that the nonabsorbable disaccharides exert not one but several effects that may benefit patients with this condition; these effects include laxative properties and effects on ammonia production and metabolism (3, 4). The beneficial action of lactulose has been related to its effectiveness in lowering blood ammonia levels (5, 6). One hypothesis that has been refuted stated that because of an acid intracolonic environment large quantities of ammonia might be trapped and excreted in the stool. This seemed unlikely to be the dominant mechanism because a commensurate rise of fecal ammonia has not been found (5, 7).

However, the studies of Vince, Killingley and Wrong (8) and Vince and Burridge (9) have suggested that lactulose reduces gut ammonia production *in vitro* by increasing ammonia incorporation and diminishing ammonia production in incubated stool samples. Moreover, lactulose causes a decrease in the total body urea pool accompanied by a twofold to threefold increase in stool nitrogen (10, 11), a finding compatible with the altered ammonia metabolism by gut flora found *in vitro*. Recent studies of the *in vitro* short-chain fatty acid (SCFA) production in fecal incubations have reported major changes in the profile of SCFA production associated with the fermentation of lactulose, indicating that lactulose decreases colonic protein degradation (12, 13).

The purposes of the study were to further investigate and partly reconfirm the effects of the oral administration of increasing amounts of lactulose on fecal concentrations and excretions of ammonia, nitrogen and SCFAs *in vivo* and to investigate whether the *in vivo* administered lactulose, as it passes into the large bowel, is associated with a reduction in bacterial ammonia production in fecal incubations (*in vivo–vitro* design) in the same manner as reported by Vince, Killingley and Wrong (8) and Vince and Burridge (9), who investigated the effects of extraintestinal lactulose added directly to fecal homogenates (*in vitro–vitro* design).

MATERIALS AND METHODS

Subjects. Twelve healthy persons (4 men and 8 women; 25 to 48 yr old) volunteered to participate in the study, which was approved by the local ethics committee. The subjects were given lactulose syrup (0.66 gm/ml) twice a day for 3 consecutive days in each experimental period. Doses (0, 20, 40, 80 and 160 gm/24 hr) were doubled from each period to the next. On the last day in each period 24-hr stools were collected and either frozen or used immediately for incubation experiments. No

Received January 7, 1992; accepted July 6, 1992.

This work was supported by the Danish Medical Research Council, the Foundation of Jeppe Juhl and his wife Ovita Juhl, the Foundation of Esper Boel, the Foundation of Lundbeck, and the Danish Foundation for Advancement of Medical Science.

Address reprint requests to: Per Brøbech Mortensen, M.D., Department of Medicine A 2151, Rigshospitalet, DK-2100 Copenhagen, Denmark.

31/1/41320

TABLE 1. Fecal mass, pH, carbohydrates, sodium, potassium and osmolality (mean ± S.E.M.) in 12 subjects administered increasing amounts of lactulose (0 to 160 gm/day)

Lactulose (gm/day)	Mass (gm/day)	pH (units)	Carbohydrates		Sodium		Potassium		Osmolality (mOsm/L)
			mmol/L	mmol/day	mmol/L	mmol/day	mmol/L	mmol/day	
0	120 ± 27	6.9 ± 0.1	0 ± 0	0 ± 0	12 ± 3	2 ± 1	119 ± 6	13 ± 3	450 ± 34
20	150 ± 24	6.8 ± 0.1	0 ± 0	0 ± 0	16 ± 5	2 ± 1	111 ± 6	16 ± 2	417 ± 18
40	297 ± 46	6.7 ± 0.1	0 ± 0	0 ± 0	9 ± 3	3 ± 1	110 ± 9	35 ± 7	423 ± 20
80	464 ± 109	5.9 ± 0.2	7 ± 3	2 ± 1	26 ± 7	11 ± 3	71 ± 10	35 ± 10	420 ± 21
160	1,604 ± 127	4.9 ± 0.1	79 ± 9	128 ± 18	43 ± 8	66 ± 11	36 ± 5	57 ± 9	417 ± 27
Two-way ANOVA (0 to 160 gm/day)	< 10-5	< 10-6	< 10-6	< 10-6	< 10-3	< 10-5	< 10-5	< 10-3	0.61
Two-way ANOVA (0 to 80 gm/day)	0.01	< 10-3	0.08	0.07	0.08	0.07	< 10-2	0.07	0.79

dietary questionnaires, restrictions or recommendations were given. Antibiotics were not administered for a period of at least 2 wk before sampling.

Preparation of Homogenates. Stools were homogenized with five times their weight of sodium bicarbonate (150 mmol/L; pH 7.8) or sodium potassium chloride (Na = 100 mmol/L; K = 50 mmol/L) under oxygen-free nitrogen, creating 16.6% fecal homogenates. In incubations with added external substrates, 100 mg of pure lactulose or albumin (Merck Chemical Co., Darmstadt, Germany) were administered as dry powder to vials filled with nitrogen, before the incubations were started by the addition of 10 ml of fecal homogenate, to give final substrates concentrations of 10 mg/ml. Dispersion was performed by shaking for 1 to 2 min. In incubations without the external substrate addition 10 ml of fecal homogenate was added to the vials, which were similarly closed by screw caps after additional filling with nitrogen. Incubations were started simultaneously within 1 hr after defecation, in duplicate and at 37° C for 6 hr and 24 hr. Gas venting was not used, and hydrogen, carbon dioxide or sources of nitrogen were not added to the vials. Termination was performed by freezing of the vials, which were kept closed during the procedure and stored at − 18° C until analysis.

Analysis. Fecal suspensions were pretreated by steam distillation as described by Zijlstra et al. (14) before gas liquid chromatographic analysis of SCFAs. A 0.5-μl distilled sample was automatically injected splitless into a Hewlett-Packard 5890A gas chromatograph equipped with a wide-bore, 530-μm (internal diameter), 30-m long HP-FFAP cross-linked fused silica capillary column with a film thickness of 1 μm (Hewlett-Packard, Palo Alto, CA). Carrier and make-up gas was helium with a flow rate of 8 and 20 ml/min, respectively. Injection and flame ionization detector temperatures were 200° C. The oven temperature was 115° C for 2 min before the temperature was raised 5° C/min to 150° C. SCFA concentrations were calculated from areas of gas chromatographic peaks (internal standard was 2-ethylbutyrate) automatically calculated on a Hewlett-Packard 3396A integrator connected on-line to the gas chromatograph.

L-lactate and D-lactate were measured by the enzymatic methods using specific L-lactate and D-lactate dehydrogenases from Boehringer Mannheim (Mannheim, Germany) (15).

Ammonia was determined by applying Berthelot's indophenol reaction directly on diluted feces as described by Gips, Reitsema and Wibbens-Albersts (16), and total nitrogen was determined by the method of Kjeldahl.

Sodium, potassium and osmolality were determined on fecal water obtained from stool samples diluted with distilled water and centrifuged at 3,000 rpm for 10 min. Sodium and potassium were analyzed on Flame Photometer model 143 (Instrumentation Laboratory Inc., Boston, MA), and osmolality was determined by freezing-point depression on a Micro-Osmometer model 3MO (Advanced Instruments Inc., Needham Heights, MA).

Carbohydrates were determined as fecal water–reducing substances by the semiquantitative Clinitest (Ames Division of Miles Laboratories, Elkhart, IN) (17). In control experiments with the addition of saccharide to fecal samples, the method proved to detect even small amounts of glucose, galactose, fructose, lactose and lactulose but could obviously not distinguish between them.

Measurements of fecal pH were accomplished with a glass electrode (Radiometer GK 2401C) connected to a Radiometer research pH meter (PHM64, Radiometer, Copenhagen, Denmark).

Statistics. Statistical analysis was a two-way ANOVA (Friedman's paired-analysis of variance) and was performed on a IBM PS/50 personal computer.

RESULTS

Table 1 shows the stool mass, pH, osmolality and concentrations and excretions of carbohydrates and electrolytes. These data were consistent with other investigations in that stool mass increases, pH decreases and osmolality were unchanged as the dose of lactulose increased (7). When the fermentation capacity was exceeded, carbohydrate was excreted and the decrease in the sum of sodium and potassium, equal to the increase in the cation gap, was 52 mmol/L (i.e., the classic sign of an osmotic diarrhea).

Table 2 illustrates the relationship between the amount of orally administered lactulose and the concentrations and excretions of stool ammonia and total nitrogen. The concentrations of ammonia decreased as the dose of lactulose exceeded 80 gm/day, but the excretions still increased 3-fold, from 6.2 to 17.8 mmol/24 hr, because of a more than 10-fold increase in stool mass. However, up to doses of 80 gm/day, neither concentrations nor excretions of ammonia were significantly altered ($p = 0.27$ and $p = 0.14$). Concentrations of total nitrogen were also decreased threefold, from 1,043 to 300 mmol/L, as a function of lactulose intake, but again the elevation in stool mass gave the final result

TABLE 2. Fecal concentrations and excretions of ammonia and total nitrogen (mean ± S.E.M.) in 12 subjects administered increasing amounts of lactulose (0 to 160 gm/day)

Lactulose (gm/day)	Ammonia		Total nitrogen	
	mmol/L	mmol/day	mmol/L	mmol/day
0	54.5 ± 7.9	6.2 ± 1.8	1,043 ± 78	111 ± 21
20	41.4 ± 4.6	5.8 ± 0.9	943 ± 71	131 ± 19
40	39.6 ± 3.7	12.3 ± 3.0	1,000 ± 75	308 ± 66
80	39.9 ± 12.8	15.8 ± 6.2	707 ± 99	328 ± 109
160	11.3 ± 2.6	17.8 ± 4.3	300 ± 136	457 ± 113
Two-way ANOVA (0 to 160 gm/day)	< 10-4	< 10-2	< 10-6	< 10-3
Two-way ANOVA (0 to 80 gm/day)	0.27	0.14	< 10-3	0.02

TABLE 3. Production of ammonia (mean ± S.E.M.) in 16.6% fecal homogenates from 12 subjects administered increasing amounts of lactulose (0 to 160 gm/day) incubated 6 and 24 hr

Lactulose ingested (gm/day)	Ammonia production after 6 hr (mmol/L)			Ammonia production after 24 hr (mmol/L)		
	NaKCl	$NaHCO_3$	Lactulose[a]	NaKCl	$NaHCO_3$	Albumin[b]
0	7.2 ± 1.3	4.8 ± 0.9	−9.7 ± 1.8	12.7 ± 1.9	11.2 ± 2.3	+127.6 ± 55.4
20	5.5 ± 1.0	1.6 ± 0.7	−9.6 ± 1.7	13.6 ± 1.8	9.4 ± 1.6	+54.6 ± 10.2
40	5.3 ± 1.1	2.3 ± 1.1	−11.7 ± 1.4	13.0 ± 2.1	9.4 ± 2.2	+48.9 ± 11.3
80	1.9 ± 1.5	0.3 ± 1.6	−6.5 ± 2.1	7.1 ± 2.9	4.4 ± 2.3	+27.7 ± 7.7
160	−0.4 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1	0.2 ± 0.3	0.8 ± 0.3	+0.5 ± 0.3
Two-way ANOVA	< 10-4	< 10-3	0.01	< 10-4	< 10-3	< 10-3

NaKCl = unbuffered homogenates diluted with isotonic NaKCl; $NaHCO_3$ = buffered homogenates buffered with isotonic $NaHCO_3$.
[a]The reduction in ammonia production after the *in vitro* addition of 10 mg/ml lactulose to the unbuffered homogenates.
[b]The additional ammonia production after the *in vitro* addition of 10 mg/ml albumin to the unbuffered homogenates.

that stool excretions of nitrogen increased fourfold, from 111 to 457 mmol/24 hr. The percentages of ammonia were constantly 4% to 5% of total nitrogen only, irrespective of the dose of lactulose.

Table 3 reports the 6-hr and 24-hr production of ammonia in the 16.6% fecal homogenates as the oral administration of lactulose increased. In the unbuffered assays diluted with sodium potassium chloride, the initial pH at incubation start was close to that reported in Table 1 and declined with increasing lactulose doses to pH 5.6, 5.5, 5.6, 4.7 and 3.4, respectively, after 24 hr of incubation and approximately 0.2 pH units less after 6 hr. In the bicarbonate-buffered assays, pH was within 7.0 to 7.5 during the whole period of incubation. The formation of ammonia was reduced from a substantial production to negligible amounts independently of incubation time and buffering solutions. When lactulose (10 mg/ml) was added directly into the homogenates, a net consumption of ammonia was registered. However, this additional effect faded as oral doses of lactulose were increased and carbohydrates eventually appeared in the feces. On the other hand, ammonia production could be increased 10-fold by the addition of albumin (10 mg/ml), but this protein-induced production of ammonia was also almost completely inhibited by the oral intake of lactulose.

Table 4 shows that concentrations of fecal SCFAs were rather constant and tended to decrease at high doses of lactulose, almost corresponding to the simultaneous appearance of lactate in feces. Thus total organic acid concentrations were rather constant despite the highly increased colonic production of these substances caused by the fermentation of lactulose. The influence of lactulose was reflected in the ratios of acetate, which increased from 65% to 89%; in the ratios of all other SCFAs with 3-6-carbon atoms, which correspondingly decreased from 35% to 11%; and, as mentioned, in the increases in fecal lactate from low concentrations of 2 to 4 mmol/L to 43 mmol/L. Therefore the rise in organic acid excretion was a simple function of stool mass.

Tables 5 and 6 illustrate the corresponding productions of SCFAs and lactate in the fecal incubations. SCFA production ratios and lactate production followed the pattern of the fecal concentrations from Table 4. In the buffered assays SCFA productions tended to increase as high doses of lactulose increased concentrations of fermentable carbohydrates in feces. The carbohydrates were probably also responsible for the formation of lactate (Table 6). Fermentation is known to cease as it acidifies the colonic contents (13), which might explain the unchanged formation of SCFAs in the unbuffered assays even after carbohydrate concentration in feces

TABLE 4. Fecal concentrations, excretions and ratios of SCFAs and DL-lactate (mean ± S.E.M.) in 12 subjects administered increasing amounts of lactulose (0 to 160 gm/day)

Lactulose (gm/day)	Total SCFA		C2	C3	C4	iC4-6	Lactate		SCFA + lactate	
	mmol/L	mmol/day	Percent of total SCFA				mmol/L	mmol/day	mmol/L	mmol/day
0	102 ± 12	13 ± 4	65 ± 2	14 ± 1	12 ± 1	9 ± 1	4 ± 1	1 ± 0	105 ± 13	14 ± 4
20	112 ± 13	18 ± 4	66 ± 2	14 ± 1	12 ± 1	8 ± 1	2 ± 0	0 ± 0	114 ± 12	18 ± 4
40	101 ± 40	27 ± 4	70 ± 2	12 ± 2	11 ± 1	7 ± 1	7 ± 4	2 ± 1	108 ± 12	29 ± 4
80	130 ± 11	61 ± 15	77 ± 3	8 ± 1	11 ± 1	4 ± 1	17 ± 5	9 ± 3	148 ± 11	69 ± 17
160	76 ± 11	119 ± 19	89 ± 3	4 ± 2	5 ± 1	1 ± 1	43 ± 8	61 ± 10	118 ± 15	180 ± 21
Two-way ANOVA (0 to 160 gm/day)	< 10-2	< 10-5	< 10-5	< 10-4	0.01	< 10-5	< 10-3	< 10-6	0.05	< 10-5
Two-way ANOVA (0 to 80 gm/day)	0.03	< 10-2	< 10-2	< 10-3	0.44	< 10-2	0.07	< 10-3	0.05	< 10-2

C2 = acetate; C3 = propionate; C4 = butyrate; iC4-6 = the sum of isobutyrate + valerate + isovalerate + hexanoate.

TABLE 5. Production of SCFAs (mean ± S.E.M.) in 16.6% fecal homogenates from 12 subjects administered increasing amounts of lactulose (0 to 160 gm/day) incubated 6 and 24 hr

Lactulose ingested (gm/day)	SCFA production in 6 hr[a]			SCFA production in 24 hr[a]		
	NaKCl	$NaHCO_3$	Lactulose[b]	NaKCl	$NaHCO_3$	Albumin[c]
0	16 ± 1; 55:20:15:10	18 ± 2; 59:19:11:11	+66 ± 5	28 ± 3; 45:22:19:14	35 ± 5; 52:21:13:14	+54 ± 6
20	15 ± 1; 55:21:15:9	16 ± 2; 60:21:10:9	+111 ± 12	30 ± 3; 45:22:21:11	30 ± 4; 48:25:14:13	+55 ± 9
40	14 ± 2; 57:18:16:9	16 ± 2; 58:21:12:9	+100 ± 11	26 ± 3; 45:22:22:11	31 ± 4; 48:24:16:12	+41 ± 7
80	14 ± 1; 67:13:13:7	16 ± 2; 65:18:10:8	+80 ± 13	27 ± 4; 61:17:16:7	35 ± 4; 58:19:14:8	+30 ± 6
160	18 ± 2; 95:4:1:1	28 ± 5; 94:5:0:1	+2 ± 2	24 ± 2; 96:2:1:1	49 ± 5; 81:9:9:1	+9 ± 5
Two-way ANOVA	0.10	0.11	< 10-2	0.60	0.05	0.01

NaKCl = unbuffered homogenates diluted with isotonic NaKCl; $NaHCO_3$ = buffered homogenates buffered with isotonic $NaHCO_3$; C2 = acetate; C3 = propionate; C4 = butyrate; iC4-6 = the sum of isobutyrate + valerate + isovalerate + hexanoate.

[a]Measured in millimolars per liter; ratio of C2/C3/C4/iC4-6 in percent of total SCFA.

[b]The additional SCFA production after the *in vitro* addition of 10 mg/ml lactulose to the unbuffered homogenates.

[c]The additional SCFA production after the *in vitro* addition of 10 mg/ml albumin to the unbuffered homogenates.

had increased. Additional lactulose and albumin was, as expected, metabolized to SCFAs, which was abolished by high oral doses of lactulose. This type of inhibition was also seen in lactate production from added lactulose. Lactate was not formed from albumin, which has been shown before (18).

DISCUSSION

The pathogenesis of hepatic encephalopathy is incompletely understood. However, most researchers agree that it is predominantly a metabolic/neurophysiological disorder of the brain and that toxins arising in the gut, mainly as a result of the action of enteric bacteria on dietary protein, are important in its genesis (19). Lactulose facilitates a reduction in toxin production and absorption by a combination of effects, some of which are incompletely delineated (20).

Lactulose (β-galactosido-fructose) is not hydrolyzed to its constituent monosaccharides in the small intestine after its oral administration, and, in the absence of small intestinal lactulase (21), lactulose passes unchanged into the large bowel. There it is hydrolyzed to galactose and fructose by the action of enteric bacteria (22). The monosaccharides then undergo fermentation to produce hydrogen, lactate and SCFAs (22, 23). Recent *in vitro* studies in fecal homogenates have shown that lactulose decreases the production of SCFAs that originate from bacterial polypeptide degradation (isobutyrate, valerate and isovalerate) in favor of an increased formation of acetate (12, 13). This change in the SCFA profile was confirmed in this *in vivo* study because acetate increased considerably and the longer 3-6-carbon SCFAs decreased. The toxicity of acetate is insignificant and considerably less than the longer chain SCFAs (24). The decreased production of iso-acids indicates that amino acid deamination is reduced (13), which may explain some of the reduction in the colonic ammonia production.

SCFAs, which under normal conditions are absorbed by the intestinal tract and rapidly removed by the liver (25, 26), increase in the plasma of patients with encephalopathy (27, 28). The concentrations of these products, which are neurotoxic, do not correlate with the degrees of encephalopathy (27, 28). A recent study found that even in maximal encephalopathy systemic concentrations of the nontoxic acetate constitute 80% of the

TABLE 6. Production of DL-lactate (mean ± S.E.M.) in 16.6% fecal homogenates from 12 subjects administered increasing amounts of lactulose (0 to 160 gm/day) incubated 6 and 24 hr

Lactulose ingested (gm/day)	Lactate production in 6 hr (mmol/L)			Lactate production in 24 hr (mmol/L)		
	NaKCl	$NaHCO_3$	Lactulose[a]	NaKCl	$NaHCO_3$	Albumin[b]
0	0 ± 0	0 ± 0	+24 ± 5	0 ± 0	0 ± 0	+0 ± 0
20	0 ± 0	0 ± 0	+58 ± 4	0 ± 0	0 ± 0	+0 ± 0
40	0 ± 0	0 ± 0	+55 ± 8	0 ± 0	0 ± 0	+0 ± 0
80	2 ± 1	0 ± 0	+47 ± 6	1 ± 2	0 ± 0	+0 ± 1
160	15 ± 4	17 ± 7	+2 ± 1	19 ± 4	11 ± 8	+5 ± 4
Two-way ANOVA	< 10-2	< 10-2	< 10-3	< 10-2	0.12	0.04

NaKCl = unbuffered homogenates diluted with isotonic NaKCl; $NaHCO_3$ = buffered homogenates buffered with isotonic $NaHCO_3$.
[a]The additional lactate production after the *in vitro* addition of 10 mg/ml lactulose to the unbuffered homogenates.
[b]The additional lactate production after the *in vitro* addition of 10 mg/ml albumin to the unbuffered homogenates.

SCFAs (28). C3-C6-SCFAs were indeed elevated, but serum levels were 200-fold to 1,000-fold lower than seen in specific metabolic defects before coma arises (29, 30). The administration of ammonia, mercaptans and SCFAs in combination has, in some studies, reproduced a comalike state when administered to normal rats (31, 32). However, the acid used in these studies was in fact the 8-carbon fatty acid, octanoate, which is a medium-chain fatty acid, not produced by colonic fermentation and considerably more toxic than the SCFAs (24).

Although the production of SCFAs increases considerably at oral lactulose administrations of up to 160 gm/day, stool concentrations of SCFAs tended to decline and lactate increased, resulting in rather constant concentrations of total organic acids in feces (Table 4). This suggests that SCFAs are rapidly absorbed when production increases, as indicated by colonic perfusion studies (25) and studies of ^{14}C-labeled glucose (33). The generation of hydrogen ions (H^+) from the dissociation of the organic acids results in a fall in colonic pH, which has been shown in studies using radiotelemetry to be maximal in the right colon (34). Fecal pH only decreases when sufficiently large doses are administered (Table 1). This probably means that the changes in ammonia, SCFA and lactate productions seen at the rectum/fecal level in this study require lower doses of lactulose to occur in the cecum.

Ammonia is the major toxin implicated in the pathogenesis of hepatic encephalopathy (19). The gastrointestinal tract is the main site of ammonia generation in humans, and it has long been assumed that ammonia production resulted from bacterial breakdown of several nitrogen sources, predominantly urea and residues of dietary protein, intestinal secretions and shed epithelial cells. Treatment with nonabsorbable disaccharides is associated with a reduction in circulating ammonia concentrations (5, 35-37), and this was initially ascribed to a decrease in the concentration and absorption of the ammonia cation secondary to colonic acidification (38). However, if acidification enhances the excretion of ammonia, then fecal ammonia excretions should increase, and this was indeed what happened (Table 2). Concentrations of ammonia decreased, but excretions did in fact increase threefold; however, this occurred only if excessive amounts (>80 gm/day) of lactulose were given (Table 2). This is in accordance with an earlier study, which found that stool concentrations of ammonia decreased and excretions were unchanged in three healthy men who ingested moderate doses of lactulose (30 to 50 gm/day) (7). The main point is, however, that ammonia only constitutes 4% to 5% of total fecal nitrogen, irrespective of the dose of administered lactulose (Table 2). Therefore the discussion about the excretion of ammonia that has been going on for more than two decades seems to be of minor importance from a clinical point of view. Much more important for the fecal nitrogen clearance are other sources of nitrogen, constituting more than 95% of total stool nitrogen, which more than doubled at doses of 40 gm/24 hr of lactulose and were increased fourfold at 160 gm/24 hr. This is probably a very important alternative of nitrogen elimination for the encephalopatic patient and seems to be a consequence of the bacterial handling of ammonia in the colonic contents and not caused by ammonia excretion *per se*.

It is known that the presence of carbohydrate in the gut lumen facilitates the growth of bacteria and the incorporation of nitrogen into bacterial protein (39). Vince, Killingley and Wrong (8) and Vince and Burridge (9), after a series of *in vitro* fecal incubation studies, concluded that lactulose may actively stimulate the incorporation of ammonia into bacterial protein. These studies were pure *in vitro* work in that lactulose was added directly into feces from healthy subjects. In our work it was decided to look at changes caused by the oral administration and *in vivo* colonic fermentation of lactulose. The results are consistent with the work of Vince, Killingley and Wrong (8) and Vince and Burridge (9) (Table 3). The ammonia production in 6-hr and 24-hr incubations declined to near zero or even to negative values corresponding to a complete inhibition of the production of ammonia or to a net consumption of ammonia, respectively. It cannot be excluded that pH may have some indirect effects on bacterial selection and

metabolism, but the effect of lactulose on ammonia production was not a consequence of a change in pH *per se* because buffered and unbuffered assays showed similar results.

The addition of albumin to assays increased ammonia production 10-fold, which also was completely inhibited in fecal incubations as oral doses of lactulose increased (Table 3). This indicates that the effect of lactulose may be even more important during episodes of gastrointestinal bleeding in avoiding large polypeptide degradation and ammonia formation in the colon. The actual presence of lactulose was not imperative for it to exert an effect on the ammonia metabolism, which also was found in stools from persons on low lactulose intake, where the fermentation of lactulose had been completed in more proximal parts of the colon. However, the external addition of lactulose was able to increase this effect up to the point where lactulose fermentation was incomplete and carbohydrates were lost in the feces (Table 3). Therefore it is likely that the effects on colonic ammonia metabolism are divided between an effect associated with the ongoing fermentation of lactulose and a relation to some intracolonic metabolic events gradually generated as a result of this fermentation, still effective in distal parts of the colon even after the disaccharide has been metabolized and has disappeared from the colonic contents. In fact, lactulose has been shown to change the bacterial metabolic pathways responsible for lactulose degradation and possibly population levels of some colonic bacteria (22, 39-41).

These findings are also consistent with observations made on the effects of lactulose on urea metabolism (10, 11). Under normal circumstances approximately one fourth of the body urea pool undergoes intestinal hydrolysis to ammonia, which is absorbed and then subsequently removed from the portal blood for hepatic urea synthesis. Thus a large fraction of the urea pool undergoes enterohepatic recycling in the form of ammonia. If administration of lactulose stimulates the incorporation of ammonia into bacterial protein, then fecal nitrogen excretion should increase and hepatic urea synthesis and the body urea pool should decrease. This indeed has been shown to happen (10, 11).

Several mechanisms of lactulose action apparently exist. They include the following: (a) a direct effect on ammonia production probably related to fermentation and bacterial growth; (b) an influence on the bacterial metabolism either by enzyme induction or on species selection, which continues to reduce ammonia production even after the actual fermentative production of SCFAs from lactulose ceases; (c) these effects may either be related to *de novo* bacterial protein synthesis or to a reduced degradation of nonbacterial proteins in the colonic contents (e.g., during gastrointestinal bleeding episodes); and (d) a detoxification of the SCFA production profile away from the potentially encephalopathogenic 3-6-carbon SCFAs toward the nontoxic acetate, although the systemic consequences of the simultaneous production of D-lactate and L-lactate are unknown and may carry some harmful effects after their absorption to the portal blood.

Acknowledgments: The skillful technical assistance of Jette Christiansen, Bodil Petersen and Anne Birgitte Larsen is greatly appreciated.

REFERENCES

1. Hoffman K, Mossel DAA, Korus W, Van de Kamer JH. Untersuchungen über die Wirkungsweise der lactulose (β-Galactosido-Fructose) im Darm. Klin Wochenschr 1964;42:126-130.
2. Phear EA, Ruebner B. The *in vitro* production of ammonia and amines by intestinal bacteria in relation to nitrogen toxicity as a factor in hepatic coma. Br J Exp Pathol 1956;37:253-262.
3. Hamilton J, Bass P. Cathartic properties of lactulose. In: Conn HO, Bircher J, eds. Hepatic encephalopathy: management with lactulose and related carbohydrates. East Lansing, MI: Medi-Ed Press, 1988:303-313.
4. Soeters P, van Leeuwen PAM, van Berlo CLH. Nitrogen metabolism in the gut. In: Conn HO, Bircher J, eds. Hepatic encephalopathy: management with lactulose and related carbohydrates. East Lansing, MI: Medi-Ed Press, 1988:31-39.
5. Zeegan R, Drinkwater JE, Fenton JCB, Vince A, Dawson AM. Some observations on the effects of treatment with lactulose on patients with chronic hepatic encephalopathy. Q J Med 1970;39: 245-263.
6. Elkington SG, Floch MH, Conn HO. Lactulose in the treatment of chronic portal-systemic encephalopathy. N Engl J Med 1969;281: 408-412.
7. Agostini L, Down PF, Murison J, Wrong OM. Faecal ammonia and pH during lactulose administration in man: comparison with other cathartics. Gut 1972;13:859-866.
8. Vince A, Killingley M, Wrong OM. Effect of lactulose on ammonia production in a fecal incubation system. Gastroenterology 1978; 74:544-549.
9. Vince AJ, Burridge SM. Ammonia production by intestinal bacteria: the effects of lactose, lactulose and glucose. J Med Microbiol 1980;13:177-191.
10. Weber FL Jr. The effect of lactulose on urea metabolism and nitrogen excretion in cirrhotic patients. Gastroenterology 1979; 77:518-523.
11. Weber FL Jr, Fresard KM, Lally BR. Effects of lactulose and neomycin on urea metabolism in cirrhotic subjects. Gastroenterology 1982;82:213-217.
12. Mortensen PB, Rasmussen HS, Holtug K. Lactulose detoxifies *in vitro* short-chain fatty acid production in colonic contents induced by blood: implications for hepatic coma. Gastroenterology 1988; 94:750-754.
13. Mortensen PB, Holtug K, Bonnén H, Clausen MR. The degradation of amino acids, proteins and blood to short-chain fatty acids is prevented by lactulose. Gastroenterology 1990;98:353-360.
14. Zijlstra JB, Beukema J, Wolthers BG, Byrne BM, Groen A, Donkert L. Pretreatment methods prior to gas chromatographic analysis of volatile fatty acids from faecal samples. Clin Chim Acta 1977;78:243-250.
15. Gutmann I, Wahlefeld AW. Lactate determination with lactate dehydrogenase and NAD. In: Bergmeyer HU, ed. Methods of enzymatic analysis. New York: Academy, 1974:1464-1468.
16. Gips CH, Reitsema A, Wibbens-Alberts M. Preservation of urine for ammonia determination with a direct method. Clin Chim Acta 1970;29:501-505.
17. Ameen AZ, Powell GK, Jones LA. Quantitation of fecal carbohydrate excretion in patients with short bowel syndrome. Gastroenterology 1987;92:493-500.
18. Mortensen PB, Hove H, Clausen MR, Holtug K. Fermentation to short-chain fatty acids and lactate in human faecal batch cultures: intra- and interindividual variations versus variations caused by changes in fermented saccharides. Scand J Gastroenterol 1991; 26:1285-1294.
19. Schenker S, Brady CE. Pathogenesis of hepatic encephalopathy.

In: Conn HO, Bircher J, eds. Hepatic encephalopathy: management with lactulose and related carbohydrates. East Lansing, MI: Medi-Ed Press, 1988:15-30.
20. Uribe M. Effect of lactulose on the metabolism of short-chain fatty acids. HEPATOLOGY 1990;12:1251-1252.
21. Dahlqvist A, Gryboski JD. Inability of human small-intestinal lactase to hydrolyze lactulose. Biochim Biophys Acta 1965;110: 635-656.
22. Florent CH, Florie B, Leblond A, Rautureau M, Berniér JJ, Rambaud JC. Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an *in vivo* study). J Clin Invest 1985;75:608-613.
23. Mortensen PB, Holtug K, Rasmussen HS. Short-chain fatty acid production from mono- and disaccharides in a fecal incubation system: implications for colonic fermentation of dietary fiber in humans. J Nutr 1988;118:321-325.
24. Samson FE, Dahl N Jr, Dahl DR. A study on the narcotic action of the short chain fatty acids. J Clin Invest 1956;35:1291-1298.
25. Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG. Absorption of short-chain fatty acids by the colon. Gastroenterology 1980;78:1500-1507.
26. Pomare EW, Branch WJ, Cummings JH. Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. J Clin Invest 1985;75:1448-1454.
27. Lai JCK, Silk DBA, Williams R. Plasma short-chain fatty acids in fulminant hepatic failure. Clin Chim Acta 1977;78:305-310.
28. Clausen MR, Mortensen PB, Bendtsen F. Serum levels of short-chain fatty acids in cirrhosis and hepatic coma. HEPATOLOGY 1991;14:1040-1045.
29. Hillman RE. Simple, rapid method for determination of propionic acid and other short-chain fatty acids in serum. Clin Chim 1978;5:800-803.
30. Budd MA, Tanaka K, Holmes LB, Efron ML, Crawford JD, Isselbacher KJ. Isovaleric acidemia: clinical features of a new genetic defect of leucine metabolism. N Engl J Med 1967;277: 321-327.
31. Zieve L, Doizaki WM, Zieve FJ. Synergism between mercaptans and ammonia or fatty acids in the production of coma: a possible role for mercaptans in the pathogenesis of hepatic coma. J Lab Clin Med 1974;83:16-28.
32. Zeneroli ML, Ventura E, Baraldi E, Penne A, Messori A, Zieve L. Visual evoked potentials in encephalopathy induced by galactosamine, ammonia, dimethylsulfide and octanoic acid. HEPATOLOGY 1982;2:532-538.
33. Bond JH Jr, Levitt MD. Fate of soluble carbohydrate in the colon of rats and man. J Clin Invest 1976;57:1158-1164.
34. Bown RL, Gibson JA, Sladen GE, Hicks B, Dawson AM. Effects of lactulose and other laxatives on ileal and colonic pH as measured by a radiotelemetry device. Gut 1974;15:999-1004.
35. Bircher J, Müller J, Guggenheim P, Haemmerli UP. Treatment of chronic portal-systemic encephalopathy with lactulose. Lancet 1966;1:890-893.
36. Fessel JM, Conn HO. Lactulose in the treatment of acute hepatic encephalopathy. Am J Med Sci 1973;266:103-110.
37. Patil DH, Westaby D, Mahida YR, Palmer KR, Rees R, Clark ML, Dawson AM, et al. Comparative modes of action of lactitol and lactulose in the treatment of hepatic encephalopathy. Gut 1987; 28:255-259.
38. Bircher J, Haemmerli UP, Trabert E, Largiacker F, Mocetti T. The mechanism of action of lactulose in portal-systemic encephalopathy: nonionic diffusion of ammonia in the canine colon. Rev Fr Etud Clin Biol 1971;16:352-357.
39. Lebek G, Luginbühl M. Effect of lactitol and lactulose on human intestinal flora in portal-systemic encephalopathy. In: Conn HO, Bircher J, eds. Hepatic encephalopathy: management with lactulose and related carbohydrates. East Lansing, MI: Medi-Ed Press, 1988:271-281.
40. Florent CH. Colonic metabolism of lactulose in man. In: Conn HO, Bircher J, eds. Hepatic encephalopathy: management with lactulose and related carbohydrates. East Lansing, MI: Medi-Ed Press, 1988:113-128.
41. Riggio O, Varriale M, Testore GP, Di Rosa R, Di Rosa E, Merli M, Romiti A, et al. Effect of lactitol and lactulose administration on the fecal flora in cirrhotic patients. J Clin Gastroenterol 1990;12: 433-436.