Zeitschrift für
Ernährungswissenschaft
Z Ernährungswiss 33:185–194 (1994)

Dose dependency of fermentation and the extent of renal excretion of Palatinit (Isomalt) in rats with respect to its energy value

H. Herfarth[1], L. Klingebiel[2], N.C. Juhr[3] and R. Grossklaus[2]

[1]Abt. Gastroenterologie, Universitätsklinikum Essen
[2]Abt. Ernährungsmedizin, Max von Pettenkofer-Institut des Bundesgesundheitsamtes Berlin
[3]Zentrale Tierlaboratorien der Freien Universität Berlin

Zur Dosisabhängigkeit der Fermentation und dem Ausmaß der renalen Ausscheidung von Palatinit (Isomalt) bei Ratten in Hinsicht auf seine energetische Bewertung

Summary: The impact of dose-dependent caloric salvage by microbial fermentation processes in the lower gut and the extent of renal excretion for the overall energetic availability of the alternative bulk sweetener Palatinit were investigated in rats.

To evaluate the extent of dose-dependent fermentation a conventional and a germ-free rat model were used and fecal excretions of Palatinit after intragastric application were compared. Because of the lack of bacterial colonization in the gastrointestinal tract in germ-free rat the difference in fecal excretion of Palatinit between germ-free and conventional rat is mainly due to bacterial fermentation. To determine the amount of renal excretion of Palatinit the urine was collected.

The experiments were conducted using different amounts of Palatinit (300 and 1200 mg/kg body weight = mg/kg b.w.). Fecal excretions of Palatinit and its monomers (sorbitol and mannitol) were measured by high-performance liquid chromatography (HPLC) and for the determination of renal excretions a gas chromatography system was used.

After the application of 300 mg/kg b.w. Palatinit only the breakdown product sorbitol could be recovered in the feces of germ-free rats (29 % of the applied dose). No intact Palatinit could be found. In contrast, neither Palatinit nor the breakdown products sorbitol or mannitol could be detected in the feces of conventional rats after application of the same dose. After the application of the higher dose only small amounts of intact Palatinit were found in the feces of germ-free rats (average 12 %). There was no intact measurable Palatinit in the feces of conventional rats. The fecal excretions of sorbitol and mannitol in the feces of the germ-free rats were 55 % and 39 %; in conventional rats only 21 % sorbitol was excreted.

Only traces of Palatinit, sorbitol or mannitol were found in the urine of conventional and germ-free rats after application of the low as well as the high dose.

In conclusion, this study clearly shows the dose dependency of fermentation and therefore the dose dependency of the energetic (i.e., caloric) availability of this disaccharide sugar alcohol. In the calculation of the energy value of Palatinit the renal excretion of Palatinit and its monomers can be neglected.

Abbreviations:

b.w.:	Body weight	MSTFA:	N-methyl-N-trimethylsilyltrifluor-acetamide
GPM:	α-D-glucopyranosyl-1,6 mannitol	SCFA:	Short-chain fatty acids
GPS:	α-D-glucopyranosyl-1,6 sorbitol	SD:	Standard deviation
I.D.:	Internal diameter		

Zusammenfassung: Bei Ratten wurde die Dosisabhängigkeit der kalorischen Nutzung durch mikrobielle Fermentationsprozesse im Dickdarm sowie das Ausmaß der renalen Ausscheidung des Zuckeraustauschstoffes Palatinit in Hinsicht auf seine energetische Bewertung untersucht.

Anhand eines konventionellen und eines keimfreien Rattenmodells wurde das Ausmaß der Dosisabhängigkeit der Fermentation bewertet. Hierbei wurden die Ausscheidungen vom Palatinit in den Faeces nach intragastraler Applikation verglichen. Da bei keimfreien Ratten jegliche bakterielle Besiedlung des Gastrointestinaltraktes fehlt, stellt die Differenz der Ausscheidung von Palatinit in den Faeces den Umfang der bakteriellen Fermentation dar. Während der Versuchsperiode wurde der Urin der Ratten gesammelt, um die renale Ausscheidung des Palatinit zu bestimmen.

Die Experimente wurden mit zwei unterschiedlichen Palatinitdosierungen durchgeführt (300 und 1200 mg/kg Körpergewicht = mg/kg KG). Die Ausscheidung von Palatinit in den Faeces wurde mittels eines HPLC (high-performance liquid chromatography) Systems bestimmt. Das Ausmaß der Ausscheidung von Palatinit über die Niere wurde mittels Gaschromatografie gemessen.

Nach der Verabreichung von 300 mg/kg KG Palatinit konnte nur das Abbauprodukt Sorbit in den Faeces keimfreier Ratten nachgewiesen werden (29 % der verabreichten Gesamtdosis). Intaktes Palatinit war nicht meßbar. Im Gegensatz dazu konnten nach Verabreichung derselben Dosis an konventionellen Ratten weder Palatinit noch die Abbauprodukte Sorbit und Mannit in den Faeces nachgewiesen werden. Nach Applikation der größeren Dosis wurden nur kleine Mengen Palatinit in den Faeces keimfreier Ratten gefunden (durchschnittlich 12 %). Wiederum war in den Faeces konventioneller Ratten kein intaktes Palatinit messbar. Die Ausscheidung von Sorbit und Mannit betrug bei den keimfreien Ratten 55 % sowie 39 %, bei den konventionellen Ratten wurden nur noch 21 % der verabreichten Sorbitmenge ausgeschieden.

Im Urin von konventionellen und keimfreien Ratten waren nur noch Spuren von Palatinit, Sorbit und Mannit nachweisbar unabhängig von der applizierten Palatinitdosis.

Zusammenfassend zeigt diese Studie deutlich die Dosisabhängigkeit der Fermentation und folglich die Dosisabhängigkeit der energetischen bzw. kalorischen Nutzung dieses Disaccharidalkohols. Bei der Berechnung des Energiegehalts von Palatinit ist dessen Ausscheidung über die Nieren zu vernachlässigen.

Key words: Sugar substitute – Palatinit – energy value – fermentation – germ-free rat

Schlüsselwörter: Zuckeraustauschstoff, Palatinit, Brennwert, Fermentation, keimfreie Ratte

Introduction

The sugar alcohol Palatinit (isomalt), a mixture of the two disaccharide polyols, α-D-glucopyranosyl-1,6-sorbitol (= GPS) and α-D-glucopyranosyl-1,6-mannitol (= GPM), is a white, crystalline, sweet-tasting water soluble substance. Beside its reduced cariogenic property (19), Palatinit is designed to replace high-calorie sugars in food; it is claimed to have a lower caloric utilization when used as a sweetening agent (12, 33, 38). Palatinit is manufactured from sucrose by enzymic rearrangement to isomaltose followed by chemical reduction of the fructose unit to mannitol and sorbitol (17). Hydrolysis of Palatinit liberates glucose (50 %), sorbitol (25 %) and mannitol (25 %) (12).

Dietary oligosaccharides which are not enzymatically digested or absorbed in the small intestine are transported into the lower, microbially colonized part of the digestive tract. A part of these substances together with variable amounts of glycoproteins from intestinal secretions such as mucins are fermented by intestinal micro-organisms (2, 3, 5, 41, 43). It is postulated that Palatinit is only partially enzymatically hydrolyzed and absorbed in the small bowel and the remainder (as well as not absorbed sorbitol and mannitol) is partially fermented by the intestinal microflora (16). Since oxygen is absent in the large intestine the decomposition products are short-chain fatty acids (SCFA), lactic acid, carbon dioxide, hydrogen, and methane as well as some heat production and

growth of the fermenting bacteria (13, 24, 27). The SCFA's are absorbed rapidly and contribute to the overall caloric need of the body (6, 14, 40). A non-invasive breath test for hydrogen in the end-expiratory air has been used in the diagnosis of various types of carbohydrate malabsorption (4, 18). While measuring breath hydrogen and methane, Fritz et al. found a linear relation between a dose of 20–50 g Palatinit and exhalation of hydrogen in healthy volunteers (11).

In this study a conventional and a germ-free rat model were used to quantify the extent and the dose dependence of fermentation of Palatinit. Non-caecectomized germ-free rats were used in earlier experiments with Palatinit and other sugar polyols (28, 45–47). A longer gastrointestinal transit time of the germ-free rat in comparison with its conventional counterpart is due to the enormous caecomegaly (1, 35) which implicates a wrong measurement of enzymatic hydrolysis and absorption of the test substance in the small bowel and the caecum (44). Therefore, the germ-free rats were caecectomized before the start of experiments; they represent the germ-free analogue to the conventional rats (20).

It might be thought likely that the energy value is reduced through renal excretion of monomers of Palatinit. In one study the disaccharide alcohol maltitol is found to be excreted to an extent of 14 % of the applied dose (28). As has been reported for the rat and man so far (17, 30), Palatinit appears in small amounts in urine after oral ingestion.

There are conflicting results on the extent of energy utilization of these alternative carbohydrates, but it is postulated that the physiological energetic value of Palatinit is between 6.3–13.4 kJ/g (1.5 and 3.2 kcal/g) (9, 17, 26). Recently, Krüger and co-workers found a dose dependency of the enzymatic cleavage and absorption of Palatinit in the small bowel of rats. In conclusion, it was pointed out that it is not correct to refer to experimental results obtained with high doses when estimating the energy value for a realistic low dose range (15, 26).

This study describes the extent and the dose dependence of Palatinit fermentation in a conventional and a germ-free rat model. Together with the renal excretion of Palatinit or its monomers the implication of the results for the caloric value of Palatinit is shown.

Methods

Animals

Conventional rats: Male juvenile Wistar rats weighing 104.2 ± 7.4 g were used. Germ-free rats: Male juvenile Wistar rats (HAN: Wistar, 1970, Central Animal Laboratory, Berlin-Steglitz) weighing 157.0 ± 36.5 g were used. Three weeks before the experiments started the germ-free rats were caecectomized as described previously (21).

Before each experiment, the animals were fasted for 16 h with prevention of coprophagy by limiting lateral movement in metabolic cages, but with free access to drinking water.

Experiments: The two different doses of crystalline Palatinit (300 and 1 200 mg/kg b.w.) were dissolved in a hypotonic electrolyte solution and then administered as a 1.5 ml bolus by gastric gavage as previously described (25, 26). A control group received only a 1.5 ml bolus of the electrolyte solution. During the following 24 h the rats were placed in metabolic cages with prevention of coprophagy and free access to food and water. Feces and urine were collected. The weight of feces and the volume of urine were determined and aliquots were frozen at -20 °C until carbohydrate analysis was done.

The feces were freeze-dried at -65 °C for 16 h (GT3, Leybold-Heraeus, Cologne) and the fecal dry weight determined. The feces were pulverized (Fritsch Pulverisette) and 200 mg were dissolved in 10 ml aqua dest. 250 µg ribitol as internal standard were added to an aliquot of 750 µl. The measurement of Palatinit and its monomers in the feces was performed by a HPLC procedure (23). The determination of free glucose in the feces of rats was not possible with this method. In the control animals there was an unidentified peak after a retention time of 16.30 min interfering with the retention time of free glucose. Before analysis an additional clearance of macromolecular components in the feces of germ-free rats was done by a sephadex column (G-25M, Pharmacia). Analysis of Palatinit in the urine of conventional and germ-free rats:

1 ml of urine was centrifugated (9600 x g, 5 min) and an aliquot of 200 µl was taken. As an internal standard 100 µl phenyl-β-D-glucopyranosid (1 mg/ml) was added. The derivatization and silylization with MSTFA was performed as described earlier (12). 0.5 µl of this solution was injected onto a 30 m x 0.33 mm I.D. open tubular fused-silica capillary column, coated with OV-1701. Analyses were performed on a Carlo Erba Model 4160 gas chromatograph (Carlo Erba, Hofheim) controlled by a Perkin-Elmer model Sigma 10 B integrator (Perkin-Elmer, Überlingen). All compounds were eluted employing a temperature program: 150 °C for 5 min; 5 °C/min 150–190 °C; 10 °C/min 190–220 °C and 220 °C for 10 min. The inlet and detector temperature were maintained at 260 °C. The carrier gas was helium with a flow rate: 2 ml/min; synthetic air: 400 ml/min; H_2: 25 ml/min. The splitting ratio was 1 : 20.

The following equation according to the factorial approach (42) was used to calculate the energy value (E_w) of Palatinit:

$$E_w = [(A \times B) + (1 - A) \times 0.5] \times 16.5 \times R_e \text{ kJ/g}.$$

A = fraction absorbed from the small intestine; B = fraction utilized by the body after absorption from the small intestine; R_e = ratio of the energy content of the alcohol concerned compared with that of saccharose (for Palatinit 1.03); 0.5 = proportion of the utilizable energy available from the large intestine; 16.5 = energy content of saccharose in kJ/g. Palatinit is absorbed as glucose, sorbitol and mannitol in the ratio 2:1:1 [50%:25%:25%].

All tests were conducted according to a randomized plan. The results are presented here as a mean (± SD) of six values unless otherwise indicated. Data was analyzed by means of Student's *t*-tests, and U-tests. Statistical significance was set at a probability level of 0.05.

Results

The percentage of recovery of Palatinit (GPS, GPM) and its monomers sorbitol and mannitol in the feces of germ-free and conventional rats is shown in Table 1. There was a complete hydrolysis of GPM and GPS in the gastrointestinal tract of conventional rats after application of the low as well as the high dose of Palatinit. The monomer mannitol disappeared completely with both doses after hydrolysis of GPM. A significant recovery of sorbitol was obtained after application of 1 200 mg/kg b.w. Palatinit, whereas all free sorbitol was digested in the group with the lower dose. In comparison, there was a significant recovery of intact Palatinit (GPS, GPM) and of both monomers sorbitol and mannitol in the germ-free group, which received 1 200 mg/kg b.w. Palatinit. In contrast to the conventional group, sorbitol was detectable after application of the low dose.

Table 1. Percentage recovery of Palatinit and its monomers sorbitol and mannitol in the feces of rats 24 h after intragastric administration of different doses of Palatinit (Means ± SD)

Group	n	Dose (mg/kg b.w.)	GPS	GPM	Sorbitol	Mannitol
Conventional	6	300	0	0	0	0
rats	6	1200	0	0	26.6 ± 14.7^{a}	0
Germ-free	4	300	0	0	29.2 ± 24.9^{b}	0
rats	4	1200	$9.5 \pm 6.4^{a,c}$	$15.5 \pm 6.9^{a,c}$	55.4 ± 18.6^{c}	$38.8 \pm 9.6^{a,c}$

([a] means were significantly different $p \leq 0.01$; [b,c] means were significantly different to the mean values of the same dose in the conventional rats $p \leq 0.01$)

In comparison to conventional rats, intact GPS (9.5 % SD 6.4 %) and GPM (15.5 % SD 6.9 %) in the feces of germ-free rats were detectable after the application of 1 200 mg/kg b.w. Palatinit (Table 1), suggesting bacterial fermentation processes of intact GPS and GPM after an incomplete enzymatic hydrolysis in the small bowel (Fig. 1).

The recovery of GPS and GPM as well as of sorbitol and mannitol in the urine of conventional and germ-free rats was below 1 %. Only small amounts of Palatinit and its monomers were detectable after the application of the higher dose (Table 2).

There was no difference in the water content of the feces between the different conventional rat groups (control versus 300 versus 1 200 mg/kg b.w.), but a significant difference between conventional and germ-free rats (64.6 % SD 3.3 versus 71.6 % SD 2.8; $p \leq 0.001$).

In contrast to the intestinal cleavage (i.e., disappearance) of Palatinit after the application of 300 mg/kg b.w. in conventional rats (100 %), there was a recovery rate of 7 % of the whole dose in the germ-free animals as shown in Fig. 2. After the application of 1 200 mg/kg b.w. Palatinit this difference was highly significant (95 % intestinal cleavage in conventional rats versus 70 % in germ-free rats) (Fig. 2).

Discussion

In this study a conventional and a germ-free rat model were used to quantify the extent and dose dependency of fermentation of Palatinit in the colon. For this purpose two different doses were chosen: a low dose of realistic daily sugar intake without side-effects (300 mg/kg b.w.) and a high-dose of unrealistic sugar intake (1 200 mg/kg b.w.).

Table 2. Percentage recovery of Palatinit and its monomers sorbitol and mannitol in the urine of rats 24 h after intragastric administration of different doses of Palatinit (Means ± SD)

Group	n	Dose (mg/kg b.w.)	GPS	GPM	Sorbitol	Mannitol
Conventional	6	300	0	0	0	0
rats	6	1200	≤ 1	≤ 1	≤ 1	2.1 ± 0.5
Germ-free	4	300	≤ 1	≤ 1	≤ 1	≤ 1
rats	4	1200	1.3 ± 0.8	2.3 ± 1.4	3.4 ± 2.5	3.9 ± 2.9

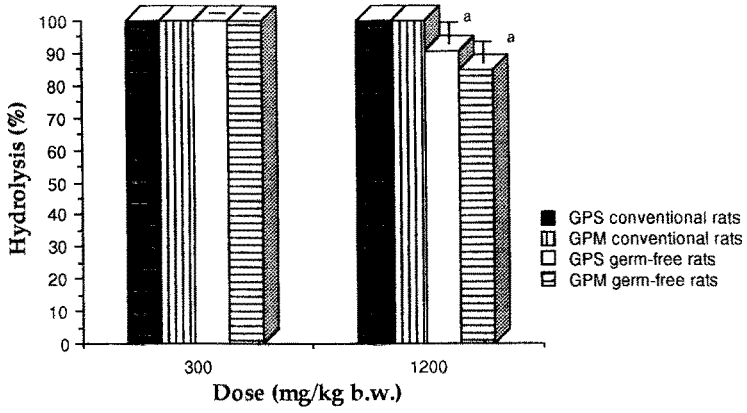

Fig. 1. Percentage of hydrolysis of GPS and GPM (± SD) in conventional and germ-free rats after intragastric administration of 300 or 1200 mg/kg b.w. Palatinit ([a] means significant different to hydrolysis of GPS and GPM in conventional rats $p \leq 0.01$).

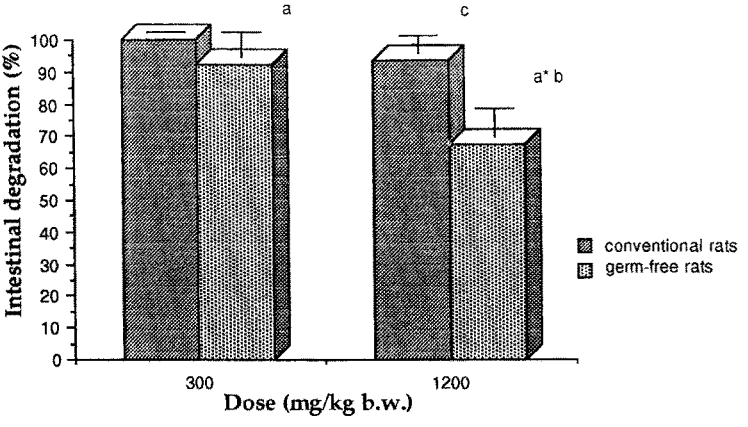

Fig. 2. Percentage of intestinal degradation of Palatinit in conventional and germ-free rats after intragastric administration of 300 or 1200 mg/kg b.w. Palatinit ([a] and [a*] means significant different to conventional rats $p^a \leq 0.01$; $p^{a*} \leq 0.001$; [b] means significant different to 300 mg/kg b.w. to germ-free rats $p^b \leq 0.02$; [c] means significant different to 300 mg/kg b.w. to conventional rats $p^c \leq 0.02$).

The amount of Palatinit excreted by the germ-free rats represents the extent of enzymatic cleavage in the small bowel because, due to the lack of intestinal bacteria, no fermentation processes could take place. Therefore, the differences in the amount of the applied substance recovered in the feces of conventional and germ-free rats represents the extent of fermentation. As shown above, only the caecectomized germ-free rat represents the analogue model to the conventional rat (20). One can postulate that after 24 h all of the applied Palatinit is excreted in the feces, because in rats the gastrocaecal transit times for sugar and sugar substitutes are 2 h at most (25).

Due to an interfering peak after the same retention time as the retention time of glucose with this HPLC system, the demonstration of free glucose in the feces was not possible. But it can be assumed that the free glucose is completely absorbed (8, 41). Krüger et al. showed a higher intraluminal disappearance of glucose than that of sorbitol and mannitol which, in contrast to the active absorption of glucose, are known to be absorbed through passive diffusion 1 h after intragastric administration of Palatinit (26). No glucose could be recovered in the feces of conventional and germ-free rats which were fed with a test diet containing a proportion of 5 % or 10 % Palatinit (39, 46, 47).

Palatinit has been shown to be fermented by the colonic flora in vivo and in vitro (11, 16, 27). The extent of this fermentation process depends on the enzymatic cleavage and absorption of Palatinit in the small bowel. An earlier study indicates the dose dependency of Palatinit hydrolysis in the small bowel. The results suggest a nearly complete hydrolysis of Palatinit in the small bowel after intragastric application of realistic low doses to rats (150 and 300 mg/kg b.w.); only about half of the dose was hydrolysed 1 h after the application of a high dose (1 200 mg/kg b.w.) (26). The recovery results of this study with the germ-free rat model, representing the digestibility of Palatinit in the small bowel because of the lack of colonic bacteria, show complete hydrolysis and absorption of the cleavage products sorbitol and mannitol after the application of the low dose.

As shown in Fig. 1, in contrast to an enzymatic hydrolysis of about 90 % of GPS and 85 % of GPM after the application of the high dose, there is complete hydrolysis of GPM and GPS after administration of the low dose of Palatinit. The higher extent of hydrolysis of GPS than that of GPM confirms earlier findings (17, 26). These results demonstrate a malabsorption of Palatinit in the small intestine due to the low affinity of the intestinal disaccharidases for this substrate (17, 30). Compared to the recovery data of Palatinit in the feces of germ-free rats, no GPS or GPM could be detected in the feces of conventional rats, indicating complete cleavage by fermentation.

After the hydrolysis of GPM and GPS there is significantly better intestinal cleavage of mannitol than of sorbitol. However, the absorption of mannitol was not complete at the higher dose in germ-free rats (see Table 1). This supports a dose dependency in the intestinal absorption and fermentation of mannitol and sorbitol. In earlier findings there are contrary results postulating a dose-dependent absorption in the small bowel of human beings (31) or describing mannitol as a non-absorbable substance (10). An intestinal absorption of 25 % (36) or at least 75 % (32) has been reported as well.

The results in Fig. 2 indicate a dose dependence of fermentation, showing significantly better intestinal cleavage of Palatinit and its monomers after application of the low dose than after the high dose of Palatinit. Fritz et al. showed a linear relation between a dose of 20 to 50 g Palatinit (~ 300–700 mg/kg b.w. Palatinit contained in a diet) ingested by 11 normal individuals and exhalation of hydrogen (11). But with this indirect measurement of fermentation the absolute amount of Palatinit degraded in the

human colon could not be assessed with certainty. The excretion data of the germ-free rats show that in physiological doses a complete hydrolysis of Palatinit in the small bowel takes place and only sorbitol reaches the colon and is fermented by the intestinal bacteria. After ingestion of an exceedingly high dose there is a significant rise of fermentation of Palatinit and its breakdown products. This is mainly due to the reduced enzymatic hydrolysis as mentioned above and an additional inhibition of passive or active absorption of the cleavage products by osmoregulation as reported by Lorenz et al. (26, 29).

Negligible amounts of Palatinit and its monomers were present in the urine, indicating complete hydrolysis of the orally administered disaccharide polyol (Table 2). Excretions of about 37 % in germ-free animals, mainly as sorbitol and mannitol, were reported in an earlier study (47). These results support the data of Kirchgessner et al. (22) and Grupp et al. (17), who found only traces of Palatinit and its monomers in the urine of rats. In experiments with ^{14}C-labeled isomalt, 3.6 % of the administered dose was recovered in the urine of conventional rats (9).

With respect to the results we can calculate by factorial approach that 93 % of the applied low dose of 300 mg/kg b.w. undergoes hydrolysis and subsequent absorption of the cleavage-products glucose (50 %) sorbitol (18 %) and mannitol (25 %) in the small intestine. Only a small amount of the whole low dose in form of sorbitol (7 %) reaches the colon and is completely fermented, since nothing is left in conventional rats. In a high-dose range (1 200 mg/kg b.w.), 70 % of the applied dose is cleaved by hydrolysis and absorbed in the small bowel (glucose 44 %; sorbitol 11 %; mannitol 15 %), 25 % (glucose 6 %; sorbitol 9 %; mannitol 10 %) by fermentation and only sorbitol, representing 5 % of the applied dose, is excreted in the feces. According to the factorial approach in the estimation of the energy value of sugar alcohols (42), the energy losses during the decomposition of sugar alcohols are around 50 %. Taking this into account and calculating that the energetic yield of the absorbed mannitol is about 50 % (42), the overall energetic availability of Palatinit in a realistic low dose range is at least 14.3 kJ/g (3.4 kcal/g).

The polyols were administered in liquid form to fasting animals, as is generally the procedure in such studies (25, 28, 37). In these conditions, the transit time is more rapid than when they are taken up in a solid meal (7, 34) and one could expect a lower digestibility of Palatinit. However, the energetic availability of Palatinit after application of 1 200 mg/kg b.w. is calculated at 12.7 kJ/g (3.0 kcal/g). This lower energy yield is mainly due to a lower digestibility of Palatinit in the small bowel, with the consequence that a higher fraction of uncleaved Palatinit enters the lower gut. With increasing doses, Palatinit and other sugar alcohols exert an osmotic effect leading to an altered net water movement in the gastrointestinal tract and enhanced transit through the small intestine (25, 29). This leads to a reduction in the caloric availability, despite an increase of fermentation in the lower gut. But a further improvement of the fermentation processes can be assumed after an adaptation period of the caecal flora (16).

In summary, the results presented in this study strongly suggest a dose dependency of fermentation. Due to a lower cleavage of Palatinit in the small intestine after application of inappropriate high doses and, despite an increasing rate of fermentation in the lower gut, the energetic availability is clearly underestimated. Therefore, it is not permissible to calculate the overall caloric availability of Palatinit with experimental data obtained in an unrealistic high-dose range as was done in earlier studies (17, 39, 46, 47).

With respect to the energy yield of Palatinit the renal excretions can be neglected. In conclusion, the energetic value of Palatinit can be assumed to be 14.3 kJ/g (3.4 kcal/g).

Acknowledgments

This study was funded by the Deutsche Forschungsgemeinschaft (grant GR 901/1- 1).

References:

1. Abrams GD, Bishop JE (1967) Effect of normal microbial flora on gastrointestinal motility. J Proc Soc Exp Biol Med 126:301–304
2. Bond JH, Currier BE, Buchwald H, Levitt MD (1980) Colonic conservation of malabsorbed carbohydrate. Gastroenterology 78:444–447
3. Bond JH, Levitt MD (1976) Fate of soluble carbohydrate in the colon of rats and man. J Clin Invest 57:1158–1164
4. Bond JH, Levitt MD (1977) Use of breath hydrogen in the study of carbohydrate absorption. Am J Dig Dis 22:379–382
5. Caspary WF (1983) Bedeutung des Kolons als Energieverwerter. Dtsch Med Wochenschr 108:713–716
6. Cummings JH, Englyst HN, Wiggins HS (1986) The role of carbohydrates in lower gut function. Nutr Rev 44:50–54
7. De Vries JJ, Collin T, Bijleveld MA, Kleibeuker JH, Vonk RJ (1988) The use of complex carbohydrates in barley groats for determination of the mouth-to-caecum transit time. Scand J Gastroenterol 23:905–912
8. Dills WL (1989) Sugar alcohols as bulk sweeteners. Ann Rev Nutr 9:161–186
9. Figdor SK, Allingham RP, Kita DA, Hobbs DC (1987) Caloric utilization of sorbitol and isomalt in the rat. J Agric Food Chem 35:996–1001
10. Fordtran JS, Rector FC, Ewton MF, Soter N, Kinney J (1965) Permeability characteristics of the human small intestine. J Clin Invest 44:1935–1944
11. Fritz M, Siebert G (1985) Dose dependence of breath nitrogen and methane in healthy volunteers after ingestion of a commercial disaccharide mixture Palatinit. Br J Nutr 54:389–400
12. Gau W, Kurz J, Müller L, Fischer E, Steinle G, Grupp U, Siebert G (1979) Analytische Charakterisierung von Palatinit. Z Lebensm Unters Forsch 168:125–130
13. Goodlad RA, Ratcliffe B, Fordham JP, Wright NA (1989) Does dietary fibre stimulate intestinal epithelial cell proliferation in germ-free rats? Gut 30:820–825
14. Grossklaus R (1983) Energy gap. Nutr Res 3:595–604
15. Grossklaus R (1987) Dosisabhängigkeit der energetischen Nutzung von Zuckeraustauschstoffen. Dtsch Zahnärztl Z 42:S154–S158
16. Grossklaus R, Klingebiel L, Lorenz S, Pahlke G (1984) Risk-benefit analyses of new sugar substitutes: 2. The formation of short chain fatty acids in the ceca of adapted and non-adapted juvenile rats. Nutr Res 4:459–468
17. Grupp U, Siebert G (1978) Metabolism of hydrogenated palatinose, an equimolar mixture of α-D-glucopyranosido-1,6-sorbitol and α- D-glucopyranosido-1,6-mannitol. Res Exp Med 173:261–278
18. Hepner GW (1978) Breath tests in gastroenterology. Adv Int Med 23:25–45
19. Hoeven van der JS (1980) Cariogenicity of disaccharide alcohols in rats. Caries Res 14:61–66
20. Juhr NC (1975) Der mikrobielle Faktor im Ernährungsversuch. Einfluß von Koprophagiebehinderung und Cäcektomie bei keimfreien und konventionellen Ratten. Tierlaboratorium 2:173–207
21. Juhr NC (1971) Eine einfache Vorrichtung für Eingriffe an gnotobiotischen Versuchstieren. Z Versuchstierkd 13:177–178
22. Kirchgessner M, Zinner PM, Roth H-P (1983) Energiestoffwechsel und Insulinaktivität bei Ratten nach Palatinitfütterung. Internat J Vit Nutr Res 53:86–93
23. Klingebiel L, Krüger D, Grossklaus R (1990) Analysis of Palatinit (isomalt) and its monomers in rat intestinal samples by high-performance liquid chromatography with ultraviolet detection. J Chromatogr Biomed Appl 527:238–243
24. Krüger D, Grossklaus R (1991) In vitro energy conversion of sugars and sugar substitutes by rat caecal flora. Thermochim Acta 193:173–181
25. Krüger D, Grossklaus R, Herold M, Lorenz S, Klingebiel L (1992) Gastrointestinal transit and

digestibility of maltitol, sucrose and sorbitol in rats: a multicompartmental model and recovery study. Experientia 48:734–740
26. Krüger D, Grossklaus R, Klingebiel L, Ziese T, Koch-Gensecke S (1991) Caloric availability of Palatinit in the small intestine of rats: Implications of dose dependency on the energy value. Nutr Res 11:669–678
27. Krüger D, Grossklaus R, Wesolowski T, Beier M (1987) Microcalorimetric investigation into the metabolic activity of the rat caecal flora in the presence of different sugars and sugar substitutes. Microbios 51:43–61
28. Lian-Loh R, Birch GG (1982) The metabolism of maltitol in the rat. Br J Nutr 48:477–488
29. Lorenz S, Grossklaus R (1984) Risk-benefit analyses of new sugar substitutes: 1. Nutritional-physiological investigations on the osmotic effect and release of glucose in juvenile rats. Nutr Res 4:447–458
30. Musch K, Siebert G, Schiweck H, Steinle G (1973) Ernährungsphysiologische Untersuchungen mit Isomaltit an der Ratte. Z Ernährungswiss Suppl 15 (3):3–16
31. Nasrallah SM, Iber FL (1969) Mannitol absorption and metabolism in man. Am J Med Sci 258:80–88
32. Pappenheimer JR (1990) Paracellular intestinal absorption of glucose, creatinine and mannitol in normal animals: relation to body size. Am J Physiol 259:G290–G299
33. Paulus K (1980) Zuckerersatzstoffe: Anforderungen und Eigenschaften am Beispiel Palatinit. ZFL 31:128–132
34. Read NW, Cammack J, Edwards C, Holgate AM, Cann PA, Brown CV (1982) Is the transit time of a meal through the small intestine related to the rate at which it leaves the stomach? Gut 23:824–828
35. Sacquet E, Garnier H, Raibaud P (1970) Etude de la vitesse du transit gastro-intestinal des spores d'une souche thermophile stricte de bacillus subtilis chez le rat holoxénique, le rat axeénique, le rat axénique caecectomisé. C r Séanc Soc Biol 164:532–537
36. Saunders DR, Wiggins HS (1981) Conservation of mannitol, lactulose, and raffinose by the human colon. Am J Physiol 241:G397–G402
37. Schell-Dompert E, Siebert G (1980) Metabolism of sorbitol in the intact organism. Hoppe-Seyler's Z Physiol Chem 361:1069–1075
38. Schiweck H (1980) Palatinit-Herstellung, technologische Eigenschaften und Analytik Palatinithaltiger Lebensmittel. Alimenta 19:5–16
39. Siebert G, Ziesenitz SC (1985) Dickdarmfunktionen und Energieausbeute aus Palatinit. I. Versuche an keimfreien Ratten. In: Wenk C, Kronauer M, Schutz Y and Bickel H (eds) Die Verwertung der Nahrungsenergie durch Mensch und Tier. Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp 135–137
40. Soergel KH (1982) Absorption of fermentation products from the colon. In: Kasper H, Goebell H (eds) Colon and nutrition. MTP Press Ltd, Lancaster, pp 27–34
41. Stephen AM, Haddad AC, Phillips SF (1983) Passage of carbohydrate into the colon. Gastroenterology 85:589–595
42. Voedingsraad (1987) The energy value of sugar alcohols. Voeding 48:357–365
43. Wiggins HS (1984) Nutritional value of sugars and related compounds undigested in the small gut. Proc Soc 43:69–75
44. Wostmann BS (1981) The germ-free animal in nutrition studies. Ann Rev Nutr 1:257–279
45. Würsch P, Koellreutter B, Getaz F, Arnaud MJ (1990) Metabolism of maltitol by conventional rats and mice and germ-free mice, and comparative digestibility between maltitol and sorbitol in germ-free mice. Br J Nutr 63:7–15
46. Ziesenitz SC, Siebert G (1987) The metabolism and utilization of polyols and other bulk sweeteners compared with sugar. In: Grenby TH (ed) Developments in sweeteners. Elsevier Applied Science, London, New York, pp 109–149
47. Ziesenitz SC (1986) Zur Verwertung des Zuckeraustauschstoffes Palatinit im Stoffwechsel. Beitr Infusionsther 16:120–132

Received November 4, 1993
accepted April 2, 1994

Authors' address:

Dir. u. Prof. Dr. R. Grossklaus, Leiter der Abteilung Ernährungsmedizin, Max von Pettenkofer-Institut des Bundesgesundheitsamts, Postfach 33 00 13, 14191 Berlin