Dose dependence of breath hydrogen and methane in healthy volunteers after ingestion of a commercial disaccharide mixture, Palatinit®

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1. Breath hydrogen and methane were determined by gas chromatography in eleven normal individuals given a low-fibre, mixed diet (control) and after ingestion of 20–50 g Palatinit®/d, an equimolar mixture of D-glucosyl-α(1 → 1)-D-mannitol and D-glucosyl-β(1 → 6)-D-glucitol (Isomalt®).

2. A linear relation was found (r 0.85; P < 0.001) between the amount of Palatinit ingested and breath H₂ per 10 h in subjects who did not exhale methane. If methane was formed in addition to H₂, the sum of both gases followed a linear dose-effect relation.

3. The mouth-to-caecum time, indicated by the first increase in breath H₂ after ingestion, was shortened by about half, yet no sign of diarrhoea was observed. Stool weight and stool frequency did not change significantly.

4. The linear relation between a dose of 20–50 g Palatinit and exhalation of H₂ (eventually plus methane) indicated that a relatively constant fraction of the dose given underwent cleavage and absorption in the small intestine, the remainder being transported into the large bowel. Microbial gas formation in the colon as well as the fractional transfer of these gases into the expiratory air occurred at fixed proportions, thus allowing an insight into colonic microbial contributions to carbohydrate utilization in the human large bowel.

Dietary oligosaccharides, not digested in the small gut (Wiggins, 1984), as well as small amounts of digestible carbohydrates like sucrose (Bond et al. 1980), fructose (Ravich et al. 1983) and starch (Anderson et al. 1981; Levine & Levitt, 1981; Feibusch & Holt, 1982) are transported into the lower, microbially colonized part of the digestive tract in healthy individuals. Together with dietary fibre (Cummings, 1984), variable amounts of glycoproteins from the intestinal secretions (Allen, 1981; Perman & Modler, 1982) mix in the colon with the above-mentioned dietary components to form the carbohydrate pool on which intestinal micro-organisms thrive by anaerobic fermentation (Cummings, 1983; Hungate, 1984). However, the amount of fermentable carbohydrates in the upper human colon is not precisely known. In an attempt to characterize and quantify microbial fermentations in the large bowel, we used the sugar substitute Palatinit® as a chemically well-defined material whose mammalian metabolism has been thoroughly investigated (Grupp & Siebert, 1978; Gau et al. 1979; Ziesenitz, 1983), including some aspects of its microbial utilization in the large intestine.

Palatinit, an equimolar mixture of D-glucosyl-α(1 → 1)-D-mannitol and D-glucosyl-α(1 → 6)-D-glucitol (Isomalt®), is reported to undergo small-intestinal digestion in ambulatory ileostomy patients by about 40% (Kroneberg et al. 1979), the remainder, together with non-absorbed D-mannitol and D-glucitol (D-sorbitol), being fermented by the intestinal microflora (Grupp & Siebert, 1978; Schnell-Dompert & Siebert, 1980; Ziesenitz, 1983). End-products of fermentation include hydrogen and methane which, at a certain proportion,
diffuse from the colon into the blood and are thus detectable in the expired air (Calloway & Murphy, 1968).

About 44% of the adult human population produce methane (Bjørneklett & Jenssen, 1982), which is formed by reduction of carbon dioxide with $H_2$ in methanogenic bacteria (Bryant, 1979; Doddema et al. 1979; Winter & Wolfe, 1980). Depending on the completeness of $H_2$ consumption, $CH_4$ or $H_2$, or both, are found in the expiratory air.

Frequently, the non-invasive breath test for $H_2$ in the end-expiratory air has been used in the diagnosis of various types of carbohydrate malabsorption (Bond & Levitt, 1977; Caspary, 1978; Hepner, 1978; Paige & Bayless, 1981). However, since the simultaneous determination of $CH_4$ requires more sophisticated experimental procedures, much less is known about its formation. In general, the usual breath test leads to qualitative information only (Bjørneklett & Jenssen, 1980). Since a substantial part of Palatinit is degraded microbially in the large bowel, we employed a quantitative assay system for $H_2$ and $CH_4$ in order to check whether a dose effect of Palatinit could be established on the exhalation of $H_2$ and $CH_4$, with the provision of a standardized, low-fibre diet and of an adaptation period of several days to the experimental conditions. In the present study a close correlation between the pulmonal gas excretion and the oral dose of the sugar-substitute Palatinit was established.

**EXPERIMENTAL**

*Materials*

Palatinit was a gift from Dr H. Schiweck, Obrigheim, and $\beta$-lactulose was purchased from E. Merck, Darmstadt.

*Subjects*

The investigations were carried out on eleven healthy female volunteers, aged 19–23 years. Their mean body-weight was 65.7 (SE 8.0) kg, the relative body-weight according to Broca's index was evaluated as 0.96 (SE 0.1). In preliminary trials, it was found that with a high-dietary fibre meal and a single oral dose of 10 g Palatinit, all subjects were $H_2$-responders. Apart from this ability, and the absence of any administration of antibiotics in the previous 90 d, the selection of the volunteers was randomized. Clinical check-up and laboratory values were taken before the start of the experiment; all values were found to be normal.

*Experimental design*

The volunteers received with their breakfast 200 ml orange juice and with their lunch 150 g yoghurt (control). Palatinit was added to the orange juice and the yoghurt for 1 week in a randomized order (for dosage of Palatinit, see Table 1). For purposes of calibration, two doses of 10 g $\beta$-lactulose ($D$-galactosyl-$\beta$(1 → 4)-$D$-fructose) as a non-digestible disaccharide were given at the end of a further control week. Each of the five experimental weeks (Table 1) was followed by an interval of at least 7 d.

Preliminary experiments had shown that a single dose as low as 5 g Palatinit led to a positive $H_2$ response (G. Siebert and P. Guinand, unpublished results), and that the adaptation and de-adaptation times of the $H_2$ excretion were less than 2 d (M. Fritz and G. Siebert, unpublished results). Therefore, we chose an adaptation period of 6 d in the present experiment with Palatinit, in accordance with Marthinsen & Fleming (1982), who found 5 d sufficient to establish a stable gas excretion after the addition of several dietary fibre components.

The regimen of each experimental week is detailed in Table 2. The first sample of respiratory air (see p. 392) was collected in the morning from fasted subjects and, after
Intestinal gas formation after *Palatinit®*

Table 1. **Daily doses of Palatinit®** during five experimental weeks when half was administered at breakfast with 200 ml orange juice, and half at lunch with 150 g yoghurt

<table>
<thead>
<tr>
<th>Experimental week</th>
<th>No. of subjects</th>
<th>Palatinit dose (g) on days</th>
</tr>
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<tbody>
<tr>
<td>(1) Control</td>
<td>11†</td>
<td>——</td>
</tr>
<tr>
<td>(2) Palatinit</td>
<td>11</td>
<td>20 20 20 20 20 20 20 20</td>
</tr>
<tr>
<td>(3) Palatinit</td>
<td>7 or 4</td>
<td>20 20 30 or 35 30 or 35 30</td>
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<tr>
<td>(4) Palatinit</td>
<td>7 or 4</td>
<td>20 20 30 or 35 30 or 35 40</td>
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<tr>
<td>(5) β-Lactulose</td>
<td>11‡</td>
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</table>

* An equimolar mixture of D-glucosyl-a(1 → 1)-D-mannitol and D-glucosyl-a(1 → 6)-D-glucitol (Isomalta).
† Sampling day (see Table 2).
‡ Five of the eleven subjects received additionally two 25 g doses of sucrose/d.
§ β-Lactulose, two 10 g doses.

Table 2. **Details of procedures during an experimental week**

<table>
<thead>
<tr>
<th>Experimental day...</th>
<th>1 Thu</th>
<th>2 Fri</th>
<th>3 Sat</th>
<th>4 Sun</th>
<th>5 Mon</th>
<th>6 Tue</th>
<th>7 Wed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration of Palatinit®** twice daily (Table 1)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Entries on abdominal sensations</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Standardized low-fibre diet</td>
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<td>X</td>
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<tr>
<td>Stool collection</td>
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<td>.</td>
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<td>X</td>
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<td></td>
</tr>
<tr>
<td>Analyses for hydrogen and methane in respiratory air during 10 h</td>
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<td>.</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sampling of blood for serum analyses during 5 h</td>
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<td>.</td>
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<td>X</td>
<td></td>
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</tbody>
</table>

* An equimolar mixture of D-glucosyl-a(1 → 1)-D-mannitol and D-glucosyl-a(1 → 6)-D-glucitol (Isomalta).

breakfast, in hourly intervals for 10 h. Analyses of blood serum by standard procedures for glucose, triglycerides, cholesterol and insulin were performed on fasted subjects, and after breakfast, in intervals for 5 h. Serum analysis values from subjects receiving Palatinit were not significantly different from those of the controls (values not shown).

Dietary conditions

On days 5 and 6 of each experimental week (Table 2) a standardized mixed diet was given which contained, in energy-related percentages (total 8·2 MJ), protein 16%, fat 38%, carbohydrate 46%. Dietary fibre according to Paul & Southgate (1978) was 12 g/d and lactose (only from yoghurt) amounted to 6 g/d. The last meal before taking respiratory-air samples was given at 18.00 hours on day 6, thus allowing for a fasting period of 14 h before the measurements began. The diet on day 7 was balanced in nutrients as on days 5 and 6 except that a formula drink was given for breakfast. On days 1–4 of each experimental week, and in the intervals between experimental weeks, the diet could be chosen freely with the following restrictions: no dietary-fibre supplement, no extra sugar-substitute, no laxative.
Sampling of respiratory air and analyses for $H_2$ and $CH_4$

Since $H_2$ and $CH_4$ concentrations in single expirations were very low for the simultaneous estimation with one detector (Tadesse et al. 1979), exhaled air was concentrated, under absorption of $CO_2$ with soda lime, in a spirometer (VT-3; Hellige Co., Freiburg) with an initial oxygen pressure ($P_{O_2}$) of 43 kPa. Due to the large difference in partial pressures of $H_2$ and $CH_4$ between respiratory and spirometer air, concentrations of $H_2$ and $CH_4$ increase in a linear fashion during 5 min rebreathing into the spirometer. After exactly 5 min sampling, a 5 ml sample of air was drawn from the spirometer and 2 ml injected into the gas chromatograph. The analytical set-up was as follows: GC 5720A (Hewlett Packard) with thermal-conductivity detector; carrier gas $N_2$ at 30 ml/min; two stainless-steel columns in series, 3.0 m x 3 mm i.d. and 3.6 m x 3 mm i.d. packed with molecular sieves 5A and 13X, 60/80 mesh (Supelco Inc.); oven temperature 125°C; detector temperature 150°C; bridge current 150 mA. Peak areas were evaluated in comparison with standard gas mixtures (20 and 100 $\mu$l/l each of $H_2$ and $CH_4$ in $N_2$; Linde, Munich). With a correction for the respective bronchial and spirometer volumes, determined by the helium dilution method, the volumes of exhaled $H_2$ and $CH_4$ respectively were calculated as ml or $\mu$l gas/min.

Colonic functions

Stools were collected and stool frequency was noted on days 5–7 and the wet weight of the 72 h samples determined. An additional check for the eventual formation of $CH_4$ was made by gas chromatographic analysis of 2 ml gas sampled above the stool immediately after defaecation.

Mouth-to-caecum time was estimated as described by Bond & Levitt (1975). On day 7, in each experimental week, the volunteers recorded hourly the eventual occurrence of burping, abdominal noise, fullness, meteorism, flatulence, and abdominal pain; on days 1–6, these symptoms were noted once daily.

Statistical analysis

Differences between mean values were evaluated using Student's paired $t$ test or, in the case of skewed distributions, with the Wilcoxon matched-pairs signed-rank test. Areas under the curve of the $H_2$ and $CH_4$ 10 h profiles were calculated as the sum of the hourly determined values. These 10 h values were intra- and inter-individually correlated with the doses of Palatininit ingested. Correlations were tested for significance, and regressions were compared by means of one-way ANOVA.

Ethical considerations

The purpose, nature, risks and benefits of the experiment were described to the volunteers, and they were given the opportunity to ask and to have answered all pertinent questions. Informed written consent was obtained from all subjects.

RESULTS

Exhalation of $H_2$ and $CH_4$

Mean values of $H_2$ exhaled by seven subjects who had received 20, 30 and 40 g Palatininit respectively (Fig. 1) were significantly different ($P < 0.0001$) from control values beginning with the second hour of the experiments until their completion in the evenings. The 10-h profiles after Palatininit ingestion were basically similar but higher with increasing doses of Palatininit. In the majority of experimental points (Fig. 1), $H_2$ exhalations after 20 and 40 g Palatininit differed significantly ($P < 0.05$) from those after the 30 g dose. The integral of the
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Fig. 1. Profile of breath hydrogen by non-methanogenic subjects on a low-fibre control diet (×) and with the addition of 20 g (●), 30 g (□) or 40 g (○) Palatinit®/d (an equimolar mixture of D-glucosyl-α(1→1)-d-mannitol and D-glucosyl-α(1→6)-d-glucitol (Isomalt®)). Values are means with their standard errors, represented by vertical bars, for seven subjects.

Fig. 2. Integral of 10 h total volumes of breath hydrogen in non-methanogenic subjects on a low-fibre control diet and with the addition of 20 g, 30 g or 40 g Palatinit®/d (an equimolar mixture of D-glucosyl-α(1→1)-d-mannitol and D-glucosyl-α(1→6)-d-glucitol (Isomalt®)). Mean values with their standard errors, represented by vertical bars, for seven subjects.

10 h total H₂ volume (Fig. 2) increased in proportion to the amount of Palatinit ingested. The values in Figs. 1 and 2 include only those subjects whose CH₄ exhalation was below or at the level of detectability (5 μl/l = approximately 10 μl/min).

Three persons excreted in expired air or produced in the gas phase above their fresh stools, or both, CH₄ in excess of 5 μl/l (Table 3); in these persons proportionality between
Table 3. Exhalation of hydrogen and methane (ml/10 h) after various doses of Palatinit* by three methanogenic subjects after 6 d of adaptation

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Control</th>
<th>Palatinit dose (g/d)</th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>H₂</td>
<td>CH₄</td>
<td>H₂</td>
<td>CH₄</td>
<td>H₂</td>
</tr>
<tr>
<td>1007</td>
<td>26</td>
<td>10</td>
<td>175</td>
<td>9</td>
<td>157</td>
</tr>
<tr>
<td>1010</td>
<td>2</td>
<td>34</td>
<td>59</td>
<td>61</td>
<td>161</td>
</tr>
<tr>
<td>1011</td>
<td>8</td>
<td>48</td>
<td>150</td>
<td>23</td>
<td>201</td>
</tr>
</tbody>
</table>

* An equimolar mixture of D-glucosyl-α(1 → 1)-D-mannitol and D-glucosyl-α(1 → 6)-D-glucitol (Isomalt®).
† Subject no. 1007 received 30 g/d.
‡ Subject no. 1007 received 40 g/d.
§ CH₄ was not detectable.

![Graphs](https://doi.org/10.1079/BJN19850124)

Fig. 3. Exhalation profile of (a) methane and hydrogen, and of (b) H₂ + CH₄ by a methanogenic subject on a low-fibre control diet (×) and with the addition of 20 g (○), 35 g (□) or 50 g (○) Palatinit*/d (an equimolar mixture of D-glucosyl-α(1 → 1)-D-mannitol and D-glucosyl-α(1 → 6)-D-glucitol (Isomalt®)), in comparison with the exhalation profile of (c) H₂ by a non-methanogenic subject; breakfast and lunch were served shortly after the first breath sampling and 5 h later as indicated in Fig. 1.

exhalation of H₂ and dose of Palatinit was diminished or eliminated. An example is given in Fig. 3 for one subject with elevated but almost peakless CH₄ values after Palatinit (Fig. 3(a)) and sharply raised but not dose-proportional H₂ values (Fig. 3(a)). When H₂ and CH₄ values were added, a methanogenic individual demonstrated a similar dose dependence (Fig. 3(b)) as did a non-methanogenic person (Fig. 3(c)).

Dose–effect relations

The integrals of the 10 h total volumes of H₂ (and of H₂ + CH₄ volumes, see preceding paragraph) showed a positive significant correlation with the intake levels of Palatinit. For each of the eleven subjects, individual regressions were calculated for control and three Palatinit intake values (inset, Fig. 4) and their slopes were found not to differ significantly; accordingly, a regression line covering all experiments with Palatinit may be constructed.
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Fig. 4. Dose–effect relation of the integral of the 10 h total volume of hydrogen + methane in eleven healthy volunteers on a low-fibre control diet, with 20 g β-lactulose, and with various doses of Palatinit® (an equimolar mixture of α(1 → 1)-D-mannitol and α(1 → 6)-D-glucitol (Isomalt®)). Regression line with 95% confidence interval; r 0.85; P < 0.001. Mean values are represented by horizontal bars. Inset: individual regressions.

(Fig. 4), characterized by r 0.85 (P < 0.001). It then follows that in 10 h, 6.4 (± 1.6) ml H₂ or H₂ + CH₄/g Palatinit were excreted with the respiratory air.

β-Lactulose

Exhalation of H₂ after two doses of 10 g β-lactulose (Fig. 4) was higher than after the same amount of Palatinit, as expected from the inability of human small-intestinal enzymes to cleave β-lactulose; however, the difference between mean values was not significant. H₂ or H₂ + CH₄ exhalations after β-lactulose did not significantly correlate with exhalations after any dose of Palatinit.

Mouth-to-caecum transit time

The first significant increase of H₂ exhalation above the fasting value was caused by the breakfast containing Palatinit, since mouth-to-caecum times were shorter than the breakfast-to-lunch interval of 5 h. In Fig. 5, mouth-to-caecum times after different doses of Palatinit are depicted; they differed significantly from control values but their decrease was not linear with the respective doses of Palatinit. Diarrhoea, however, was never observed under the experimental conditions of the present study.

Weight and frequency of stools

Although higher bacterial activity in the large bowel was indicated by increased H₂ production, wet weights of 72 h stools did not change significantly (Fig. 6). Stools were frequently found to be softer and of less intense colour on days 5–7 but were never watery; in the adaptation period of days 1–4 (Table 2), eight subjects reported occasional thin stools.
Fig. 5. Mouth-to-caecum transit time after formula breakfast (control) and after additional Palatinit® (an equimolar mixture of D-glucosyl-α(1 → 1)-D-mannitol and D-glucosyl-α(1 → 6)-D-glucitol (Isomalt®)). Mean values with their standard errors, represented by vertical bars. Statistical significance of differences: control v. Palatinit® $P < 0.001$; Palatinit® 10 g v. 20 g or 25 g $P < 0.05$.

Fig. 6. Stool weights (g wet weight/d) on a low-fibre control diet and with the addition of 20, 30, 35, 40 or 50 g Palatinit®/d (an equimolar mixture of D-glucosyl-α(1 → 1)-D-mannitol and D-glucosyl-α(1 → 6)-D-glucitol (Isomalt®)). Mean values with their standard errors, represented by vertical bars. Differences were not statistically significant.

Mean stool frequency was 1.1 (SE 0.2) in the controls and did not change significantly with the low, medium and high doses of Palatinit; corresponding values were 1.2 (SE 0.1), 1.0 (SE 0.2), and 1.3 (SE 0.2) respectively ($P < 0.05$; $n$ 11).

Abdominal sensations

Mean values of hourly recorded entries on day 7 of each experimental week (Table 2) are presented in Fig. 7. Abdominal noise, fullness and flatulence were more frequent with
Intestinal gas formation after Palatinit®

Fig. 7. (a) Hourly entries for 10 h (1 = mild, 2 = medium, 3 = strong) for abdominal sensations (means of eleven individuals) by volunteers on a low-fibre control diet (C), with the addition of two 10 g doses of β-lactulose (L), with the addition of two 10 g doses of Palatinit® (an equimolar mixture of D-glycosyl-α(1 → 1)-D-mannitol and D-glucosyl-α(1 → 6)-D-glucitol (Isomalt®)) (P1), two 15 g doses (n 7) or two 17.5 g doses (n 4) of Palatinit (P1I), and with the addition of two 20 g doses (n 7) or two 25 g doses (n 4) of Palatinit (P1II). (b) Sum of abdominal sensations after various doses of Palatinit.

increasing doses of Palatinit but individual plots (Fig. 7(b)) indicated considerable variability of the symptoms. When compared with Fig. 4, abdominal sensations did not coincide regularly with H₂ or H₂ + CH₄ exhalations. Even when several entries were made by the volunteers (Fig. 7), they noted their general state of health as good to very good.

DISCUSSION

Gas production in the large bowel is a ubiquitous physiological event, as generally exemplified by flatulence after certain meals and by the colonic utilization of dietary fibre; in western diets, 20–40 g fermentable carbohydrate are estimated to form the colonic pool of dietary and endogenous fermentable substrates (Cummings, 1981, 1983). With Palatinit as a representative of a whole group of nutritive sugar substitutes, colonic gas formation is enhanced (G. Siebert and P. Guinand, unpublished results). In contrast to dietary fibre material, Palatinit is a chemically well-defined substance. Thus Palatinit served in the present study both as a model compound for intestinal gas production and as the object of its more detailed investigation.

The exhalation of 6.4 (SE 1.6) ml H₂/g Palatinit in 10 h constitutes only an indirect measure of total colonic gas production. In the absence of an understanding of colonic H₂
and CH\textsubscript{4} formation (Wolin & Miller, 1983), the absolute amount of Palatinit degraded in the human colon cannot be assessed with certainty. It is thus surprising that such a close correlation between the oral dose of Palatinit contained in a whole meal and the pulmonal exhalation of H\textsubscript{2} or H\textsubscript{2} + CH\textsubscript{4} (Fig. 4) could be established. It follows from this correlation that all processes between oral intake and breath exhalation should also occur with high regularity; e.g. enzymic cleavage, small-intestinal absorption, microbial fermentation, diffusion of H\textsubscript{2} and CH\textsubscript{4} into the bloodstream and then into expiratory air are not all dose-dependent after ingestion of Palatinit but occur at constant ratios independent of the dose. There is little doubt that a regular dose dependence will also be obtained with other carbohydrates and polyols, although perhaps at different absolute values. Such a regular dose dependence has not been, to the best possible knowledge of the authors, reported in the literature, except for \(\beta\)-lactulose in aqueous solution without pre-adaptation (Bond & Levitt, 1972). In the present study, the main factors contributing to the observed regularity are the 10 h quantification of gas exhalation and the introduction of suitable dietary conditions. The established dose–effect relation (Fig. 4) also demonstrates that the fermentative capacity of the colon is not exhausted by 50 g Palatinit: with 6.4 ml H\textsubscript{2}/g Palatinit and about 14\% of the produced H\textsubscript{2} showing up in exhaled air (Levitt, 1968), 45 ml H\textsubscript{2}/g Palatinit or 2250 ml H\textsubscript{2}/50 g Palatinit per 10 h are formed in the colon. If the fermentative capacity had been exceeded, diarrhoeal symptoms via the osmotic activity of intact carbohydrates (Saunders & Wiggins, 1981) should have occurred. It should be mentioned here also that undegraded Palatinit in stools in men (Musch et al. 1973) and animals (Grupp & Siebert, 1978; Kirchgessner et al. 1983; S. C. Ziesenitz, R. Vallon, E. J. Karle, C. Benning and G. Siebert, unpublished results) has never resulted in excretion levels exceeding 1\% of the oral dose.

CH\textsubscript{4} exhalation occurs at relatively constant rates (Bond et al. 1971; Tadesse & Eastwood, 1978; Pitt et al. 1980; Tadesse et al. 1980; Bjørneklett & Jønssen, 1982), as was also observed in the present investigation (Fig. 3(a)) during 10 h when no sharp meal-related peak was found. The assumption of Wolin & Miller (1983) that CH\textsubscript{4} stems only from the fermentation of endogenous substrates cannot be entirely correct because two of our methanogenic subjects demonstrated, after 6 d of adaptation, a definite increase of CH\textsubscript{4} exhalation (Table 3), while H\textsubscript{2} exhalation alone showed little dose dependence (subjects 1010 and 1011). No qualitative alteration was observed in the present study in that methanogenic and non-methanogenic subjects stayed this way during the 15 weeks of experimentation.

Since dose-independent utilization of Palatinit in the small intestine must be inferred from these studies, and because limited small-intestinal utilization of Palatinit is one of the factors for its energy-reduced character, energy yield from Palatinit cannot depend on the dose administered within the range of the present study (20–50 g/d). H\textsubscript{2} and CH\textsubscript{4} formation from Palatinit is regarded as one of several reasons for the lowered energy yield; it may be calculated that 1 g Palatinit (\(\approx 16 \cdot 3\) kJ) giving rise to about 45 ml H\textsubscript{2} in the colon would lead to an energy loss of 3–5\% due to colonic gas (1 g H\textsubscript{2} \(\approx 143\) kJ; 45 ml H\textsubscript{2} \(\approx 0.57\) kJ), which is much less than determined by more direct methods. The discrepancy will most likely be due to Palatinit utilization by micro-organisms not producing H\textsubscript{2} and to the lack of a quantitative understanding (Wolin & Miller, 1983) already mentioned.

\(\beta\)-Lactulose was tested in the present study because Bond & Levitt (1972) proposed its use as a reference substrate in measurements of H\textsubscript{2} in exhaled air. \(\beta\)-Lactulose acts quite differently when given in aqueous solution (La Brooy et al. 1983) compared with a whole meal (Read et al. 1980), and does not give a significant correlation with the gas formed after giving glucitol (Hyams, 1983) or Palatinit (present study). The discrepancy may be due to the method of administration, the competence of different bacterial strains (\(\alpha\)- or \(\beta\)-glycosidic bonds respectively) and the absence of adaptation. However, whereas Bond & Levitt (1972)
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found 1·9 ml H₂/g β-lactulose within 2 h, it may be roughly estimated that on a per h and per g basis, 1 ml H₂ after β-lactulose would correspond to 0·6 ml H₂ after Palatinit in the present study. Since β-lactulose is exclusively used in the large bowel, our findings could be tentatively interpreted as indicating that about 60% of the Palatinit given to our volunteers was metabolized in the large bowel.

Tolerance to Palatinit in doses of up to at least 50 g/d is demonstrated in the present study (Fig. 7). It is the experience of the present authors that with any kind of overdose, e.g. by an α-glucosidase inhibitor or by excessive doses of polyols, the breath H₂ behaves quite irregularly and breath CH₄ almost disappears (M. Fritz and G. Siebert, unpublished results). In consequence, the existence of tolerance is one of the conditions which must be met for quantitative breath analysis in dose dependence. Abdominal sensations were weak enough to suggest the existence of tolerance. Other conditions are a standardized, low-fibre diet before and during breath analyses, and the adaptation of the experimental subjects by pretreatment with increasing amounts of Palatinit.

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REFERENCES

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