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On the occurrence of free glucose in the caecal contents of rats

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Summary: Free glucose was assayed in the caecal contents of rats. Whereas control animals had < 60 nmol glucose per g of caecal contents, 230 nmol glucose/g caecal contents were determined 3 hours after the administration of 300 mg Palatinit® by gastric intubation. In contrast to an earlier report (8), caecal fluid thus contained less than 3 % of the glucose concentration of 11 µmol/ml claimed to occur after Palatinit® administration by these authors (8). Since the anaerobic fermentation capacity for glucose by the caecal contents of rats amounted to at least 630 nmol glucose/min × g fresh weight at 37 °C, only the low glucose concentrations reported above are plausible.

Zusammenfassung: Der Zäkuminhalt von Ratten enthält nach 24 Stunden Fasten < 60 nmol Glucose/g; gibt man solchen Tieren 300 mg Palatinit® mit Magensonde, so steigt 3 Stunden später die Glucosekonzentration auf 230 nmol/g. Im Gegensatz zu einer früheren Literaturangabe (8), wonach 11 µmol Glucose/ml Zäkumflüssigkeit nach Palatinit®-Gaben vorkommen sollen, werden also weniger als 3 % tatsächlich gefunden. Die anaerobe Fermentationsrate des Zäkuminhalts von Ratten beträgt > 630 nmol Glucose/min × g bei 37 °C; dieser Wert macht nur die hier beschriebene niedrige Glucosekonzentration plausibel.

Key words: caecum, glucose, intestinal fermentation, Palatinit®

Introduction

Palatinit®, an equimolar mixture of D-glucosyl-α(1→6)-D-glucitol and D-glucosyl-α(1→1)-D-mannitol, is an established non-cariogenic, low-caloric sugar substitute (1). It is split only partially during the passage of the small intestine (2). Thus, more than 50 % of Palatinit® ingested arrives at the lower gut (3, 4) and will be finally degraded there through a joint attack by host and microbial activities. Intestinal micro-organisms thus play a physiological role in the utilization of Palatinit® giving rise to e.g. a reduced glucose bioavailability from this sugar substitute (5), a dose-dependent H_2-production measured in the respiratory air (6), and finally an energy loss of about 50 % (7) for the host.

In a study by Lorenz and Großklaus (8) on the contents of the intestinal tract of rats after administration of Palatinit®, it was stated that free glucose in caecal supernatants of conventional rats was near 11 µmol/ml 3 hours after administration of Palatinit®. This seemed to suggest that, in

spite of the enormous fermentative capacity of the caecal microflora, free glucose could exist in the caecum at concentrations twice that of blood sugar (5–6 µmol/ml). Such findings were surprising enough to call for a repeat of the Großklaus experiment; the outcome will be reported below.

Materials and Methods

Male Cara rats of our departmental breed, weighing 105–125 g, were kept without food for 24 h. Two animals were sacrificed with ether and 1 ml T 61*) as controls. Two animals received under light ether anaesthesia 1.5 ml 20 % Palatinit® in H_2O (300 mg/animal) by gastric intubation. After 3 hours, with ad libitum access to drinking water, these animals were sacrificed as above.

As quickly as possible, the contents of the caeca were transferred into 1.5 ml 10 % Zn-acetate and mixed immediately with 2.55 ml 0.5 M NaOH. After 15 min standing at 2 °C, 5 min centrifugations at 12.000 rpm gave clear neutral supernatants after deproteinization. Aliquots were used for the enzymatic assay of glucose *via* hexokinase with the Boehringer-Mannheim test kit. At the end of each determination of glucose, the enzymatic assay system was calibrated internally by a standard amount of glucose. The capacity of the glucose assay system was thus ensured to be sufficient in each case.

Special care was taken to follow the procedure of Lorenz and Großklaus (8) as closely as possible, with the following exceptions: (1) aliquots for glucose determinations were deproteinized as described above, while Lorenz and Großklaus (personal communication) used a Glucoquant® system destined for glucose assays in blood plasma without deproteinization: (2) Lorenz and Großklaus (personal communication) did not provide drinking water after administration of a hypertonic solution of Palatinit®; the present author maintains that access to drinking water is mandatory for a GLP experiment.

Ether narcotized male Cara rats of 142–167 g body weight were placed in an anaerobic glove box (9). Caecal contents, collected anaerobically from 3 animals, weighed 2.31 g; they were mixed with 2.88 mg glucose in 1 ml H_2O to give 5.5 µmol D-glucose/ml incubation mixture, and stirred magnetically in a thermostat at 37 °C under anaerobic conditions. Aliquots of 0.1 ml were taken during 3 hours and analysed for glucose as described above.

Results and Discussion

As shown in Table 1, less than 60 nmol glucose/g caecal content were present in control animals. This data was derived from a sensitivity limit of 10 µg glucose under the analytical conditions described above; actually, no glucose could be detected at all.

After administration of Palatinit®, 230 nmol glucose per g caecal content were found. Since caecal water could be estimated from the centrifugation step (see under Methods) to comprise about 80 % of the caecal contents and since free glucose must be assumed to exist mainly in the extracellular (aqueous) phase of the caecal contents, free glucose amounted to 290 nmol/ml in the caecal fluid.

Anaerobic fermentation (see Table 1) led to the disappearance of 14.4 µmol glucose in the first 10 min, and another 0.87 µmol in the second

*) T 61: 20 g N-Butramide, 5 g Mebezonium iodide and 0.5 g Tetracaine hydrochloride in 100 ml aqueous solution (Hoechst AG, Frankfurt).

Table 1. Glucose in caecal contents

Control value	< 60 nmol glucose/g
Palatinit® administration	230 nmol glucose/g
Fermentation rate	> 630 nmol glucose/min × g

10 min interval. Thus, the fermentation rate with glucose was > 630 nmol glucose/min per g caecal contents at 37 °C. 11 μmol glucose/ml (8) would then be fermented in < 18 min.

The existence of free glucose, arising from Palatinit®, in the suspension medium of rat caecal microorganisms should be considered under the following circumstances:

1. Glucose, once dissociated from an enzyme hydrolyzing Palatinit®, is immediately confronted in the lower gut with two high-affinity processes in competition, absorption by the caecal mucosa and fermentation by caecal bacteria. The former had to be deduced from a study of Palatinit® digestion in germ-free rats (10) and is obviously effective throughout the caecal and colonic parts of the large intestine; the latter is the overwhelming (1) event and was measured here.

2. No mechanism is known to exist for a transfer of glucose from extracaecal compartments into the caecal fluid; whatever glucose may exist in the caecal contents should stem from intracaecal metabolic processes. Neither is any metabolic mechanism known which could result in a doubling of free glucose concentrations (11 μmol/ml according to ref. [8]) over those of plasma glucose (5–6 μmol/ml).

3. The data of Table 1 demonstrate that glucose does not occur in the caecal contents of fasted rats to any appreciable extent. When 300 mg Palatinit® is given by gastric tube, about 105 mg are expected to reach the large bowel undigested (3). Since 105 mg Palatinit® contain about 50 mg glucose, about 280 μmol glucose, once liberated (see the above paragraph 1.) will eventually distribute in the caecal water. The fermentation rate of > 0.63 μmol/min × g and the unknown rate of glucose absorption through the caecal mucosa finally result in 290 nmol glucose/ml caecal fluid. This value probably represents a transient steady state during intracaecal digestion of Palatinit®.

While the glucose concentrations reported in this paper must be considered plausible, the present author is unable to confirm the data of Lorenz and Großklaus (8), so they should be taken as invalid unless they can be confirmed in an unequivocal manner.

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