Thermochimica Acta, 193 (1991) 173–182
Elsevier Science Publishers B.V., Amsterdam

In vitro energy conversion of sugars and sugar substitutes by rat caecal flora

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(Received 30 November 1990)

Abstract

A simple batch culture technique utilizing adapted rat caecal flora was used for anaerobic biodegradation of different sugars (glucose and sucrose) and sugar substitutes (sorbitol, Polydextrose® and Palatinit®) in a complex medium (thioglycollate–peptone broth). The cultures were connected to a flow calorimeter in order to quantitate bacterial heat production occurring during incubation. Moreover, increase of biomass and fermentation products (short-chain fatty acids) were measured after each 48-h experiment. If these values exceeded the results obtained from unsupplemented cultures, the calculation of the recovery of the added carbohydrate energy was based on the difference values. The results show that each test substance is converted individually to some extent into heat, biomass and fermentation acids. For all substances no significant variations were revealed regarding specific heat production. However, the specific energy conversion into biomass and metabolites varied. It is concluded that the amount of energy from microbial fermentation process available to the host organism cannot be considered constant. It may depend upon the nature or the quantity of the carbohydrate tested. In addition, the reliability of these in vitro experiments concerning the efficiency of energy conversion of nutrients by intestinal micro-organisms is discussed.

INTRODUCTION

The anaerobic degradation of certain food ingredients by the microflora of the mammalian digestive tract is a common biological process and is deeply rooted in evolution [1,2]. Diet components which are not completely absorbed in the upper regions of the gastrointestinal tract will reach the caecum and hindgut, where they serve as a fermentable substrate for anaerobic micro-organisms [3]. The main metabolites derived from soluble carbohydrate fermentation are absorbable short-chain fatty acids (SCFAs) which are believed to provide considerable metabolizable energy to the host [4,5]. Other common fermentation products which are generally considered as energy losses [6] are microbial cell mass, gases (CO_2, H_2 and CH_4) and heat.

Microcalorimetry has proven to be a useful tool for measuring the heat production of mixed bacterial cultures derived from natural ecosystems during the fermentation process, even in their natural substrates [7–11].

Controversy exists about the physiological caloric value of some new alternative carbohydrates which have been designed to replace caloric intense sugar in food (i.e. Polydextrose®, a mixture of random bound glucose polymers, and Palatinit®, a mixture of disaccharide sugar alcohols [12,13]). These compounds are described as having a reduced or no small intestinal digestibility and one has to consider that parts of the ingested amount will reach the large gut and undergo anaerobic biodegradation. Recently, attempts have been made to estimate in vivo the energetic contribution of SCFA from poorly absorbed sugar substitutes [14]. However, the extent and the rate of efficiency of fermentation is not clear. Until now, only general estimations have been made of the degree of delayed caloric availability for the host organism via absorbable microbial fermentation products [15].

The objectives of the present in vitro experiments were to evaluate the reliability of the batch culture technique in combination with flow microcalorimetry utilizing caecal flora of rats to determine the principles of the energy conversion of sugars and sugar substitutes through fermentation.

MATERIAL AND METHODS

Microflora from the caecum of juvenile male Wistar rats weighing about 100 g was used. Before each experiment, the animals were adapted to the sugar or sugar substitute by dispensing the test substance for 12 days with drinking water as a 5% solution.

The preparation of caecal content was done under a nitrogen atmosphere. Cultivation was performed with shaking for 48 h in a thioglycollate medium [7] at 37 °C without (blank) and with addition of 1% sugar or sugar substitute. The bacterial suspensions were streamed through by filtered gas (90% N_2, 5% CO_2 and 5% H_2) and pumped continuously through the measuring coil (0.7 ml volume) of an LKB 2107 Flow Microcalorimeter.

At the end of each calorimetric run the increase in biomass was calculated by direct determination of bacterial dry weight or by optical density measurements using a calibration curve. The concentrations of glucose, sorbitol, lactate and SCFAs were determined as described previously [7,14,16]. Calculations on the caloric content of metabolites were based on the values given in ref. 17. It was assumed that 1 g of each pure test substance corresponds to 16.7 kJ (4 kcal), with the exception of glucose which was defined to have 15.6 kJ g^{-1} (3.7 kcal) of energy. It was assumed that 1 g of bacterial dry weight corresponds, on average, to 20.9 kJ (5 kcal) [18,19]. Integration of the recorded calorimetric power–time curves (P–t curves) was done with an electronic integrator (Digikon, Kontron).

All experiments were repeated six times with inocula derived from six different animals; the results are presented here as the mean ($\pm$ SD) of these values. Statistical differences were checked by means of Student's t-test [20].

RESULTS

Anaerobic cultivation of adapted caecal microflora in thioglycollate broth was accompanied by the generation of P–t curves with characteristics depending on the type of previous adaptation or on the substrate added (Fig. 1). In contrast to the blank incubations of caecal flora (dotted lines in Fig. 1), the addition of sugars or sugar substitutes generally led to more profiled P–t curves (solid lines in Fig. 1) with higher and larger peaks and with substantial late heat production occurring after 10 to 25 h of cultivation.

Calculating from integral values of the P–t curves the total energy liberated in the form of heat during the 48-h incubation period, the cultures

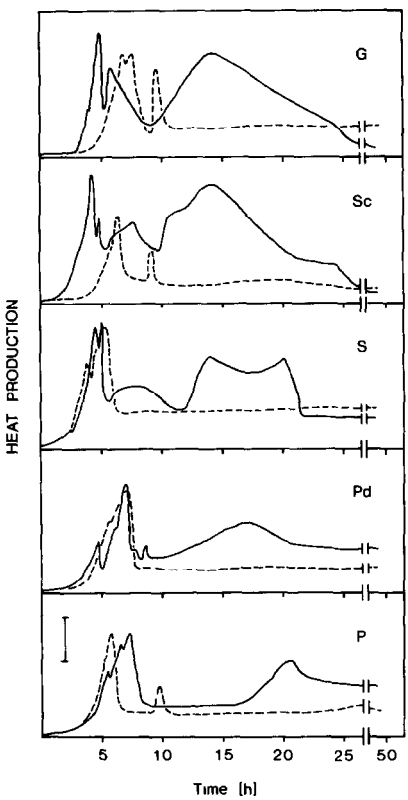

Fig. 1. Representative power–time curves generated from rat caecal microflora during anaerobic growth in a thioglycollate medium without (-----) and with the addition of sugars or sugar substitutes (———). The vertical bar represents 100 μW. G, (+)-glucose; Sc, (+)-sucrose; S, (+)-sorbitol; Pd, (+)-Polydextrose®; P, (+)-Palatinit®.

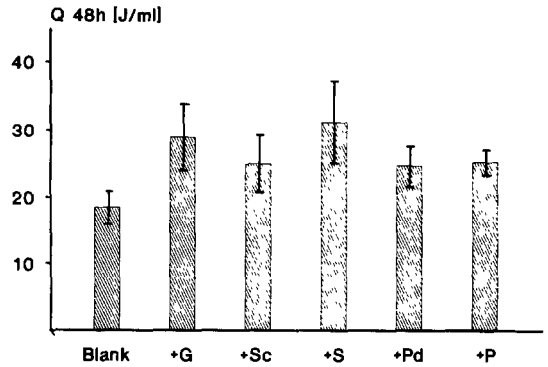

Fig. 2. Energy dissipated as heat (joule per millilitre of culture volume) by rat caecal flora during a 48-h cultivation in thioglycollate broth without (blank) and with the addition of sugars and sugar substitutes. See Fig. 1 for abbreviations.

with the carbohydrate supplement showed a significantly higher energy dissipation than did the blank medium ($p < 0.05$, Fig. 2).

The relative recovery of the gross energy of the sugars and sugar substitutes after a 48-h incubation with caecal flora is shown in Table 1.

The most substantial difference concerning the recovery of residual substrate was between Polydextrose® and Palatinit®; only about half of the Polydextrose® admixture could be degraded by the caecal flora, while more than 90% of Palatinit® disappeared from the suspensions. About 12–24% of the initial energy of the other test substances was recovered in this fraction ("residual substrate" in Table 1).

With regard to the bacterial conversion of substrate energy into heat, the lowest increase of heat evolution compared to blank experiments was found for Polydextrose®; on average, 3.7% of its gross energy content was recovered in this form. The highest percentages were noted for glucose and sorbitol tests (compare "Δ released heat" in Table 1).

Cultures containing glucose, sucrose or sorbitol as additional substrate did not exceed the biomass increase realized with pure thioglycollate broth. Conversely, Polydextrose® and Palatinit® cultures revealed a clear increase in biomass yield compared to the blanks, allowing the calculation of the fractions of energy of the sugar substitutes which were converted into cellular material. Significantly more energy in the form of biomass was recovered in the Palatinit® supplemented cultures ("Δ biomass yield" in Table 1).

A substantial portion of the caloric content was deposed in metabolites from all substrates. Most acetic acid was detected in glucose and sucrose suspensions. Lactic acid concentrations exceeding the blank values were measured only with glucose as a substrate. However, lower amounts of acetic acid were found in cultures supplemented with sugar substitutes. Propionic acid also contributed to this fraction of energy recovery when

TABLE 1

Percentage recovery of gross energy of sugars and sugar substitutes in the form of residual substrate, heat, biomass or metabolites after a 48-h incubation with rat caecal flora in a thioglycollate medium [a]

Substrate	Recovery (%)						Total recovery of gross energy (%)
	Residual substrate	Δ Released heat	Δ Biomass yield	Δ Metabolites			
				Acetic acid	Propionic acid	Lactic acid	
Glucose	12.5 ± 7.2	7.3 ± 4.8	NI	25.1 ± 8.3	NI	4.7 ± 2.7	49.5
Sucrose	23.2 ± 16.9	4.2 ± 3.2	NI	17.5 ± 9.0	NI	NI	44.9
Sorbitol	21.7 ± 10.6	7.5 ± 5.4	NI	13.2 ± 3.7 [b]	NI	NI	42.4
Polydextrose®	55.6 ± 8.0 [b,c,d]	3.7 ± 2.6	4.3 ± 1.6 [b,c,d]	6.0 ± 4.3 [b,c,d]	7.4 ± 3.4 [b,c,d]	NI	77.0
Palatinit®	7.2 ± 4.5 [c,d,e]	5.0 ± 1.6	12.5 ± 7.7 [b,c,d,e]	13.7 ± 4.3 [b,e]	1.8 ± 1.1 [b,c,d,e]	NI	40.2

[a] Δ, Data corrected for values obtained with blank thioglycollate cultures; NI, no increase versus blank. Significant differences ($p < 0.05$).
[b] Compared to glucose.
[c] Compared to sucrose.
[d] Compared to sorbitol.
[e] Compared to Polydextrose®.

TABLE 2

Specific energetic conversion of sugars and sugar substitutes into heat, biomass or metabolites (kilojoule per gram substrate fermented) calculated from recovery data exceeding the values of blank experiments in 48-h-old thioglycollate cultures of rat caecal flora [a]

Substrate fermented	Heat ($kJ\ g^{-1}$)	Biomass ($kJ\ g^{-1}$)	Metabolites (total SCFA and lactate) ($kJ\ g^{-1}$)
Glucose	1.3 ± 0.8	–	5.3 ± 2.0
Sucrose	1.0 ± 0.7	–	3.8 ± 2.0
Sorbitol	1.6 ± 1.1	–	2.8 ± 0.7 [b]
Polydextrose®	1.4 ± 1.0	1.6 ± 0.6 [b,c,d]	5.0 ± 1.8 [d]
Palatinit®	0.9 ± 0.3	2.1 ± 1.3 [b,c,d]	2.6 ± 0.9 [b,e]

[a] Significant differences b–e are as indicated in the footnotes to Table 1.

Palatinit® or Polydextrose® were tested (Table 1). Analysis for butyric, isobutyric, valeric and isovaleric acids were negative or did not exceed the blank values and are not mentioned in Table 1.

A total recovery of the energetic input of 77% was calculated for Polydextrose®, while for the other test substances the values were between 40% and 50% (Table 1).

Specific quantities were calculated for substrate energy conversion into heat and yield of biomass or metabolites (Table 2). The amount occurring as the thermal event when 1 g of substrate was fermented was of the same order of magnitude for all the sugars and sugar substitutes investigated (i.e. grand mean = 1.2 $kJ\ g^{-1}$).

Only with Polydextrose® and Palatinit® was the yield of biomass found to be higher than in the blank experiments, corresponding to 1.6 and 2.1 kJ g^{-1} substrate fermented, respectively.

The highest efficiency in substrate conversion to metabolites (SCFA or lactic acid) was found for glucose and Polydextrose®, while the lowest values were measured for sorbitol and Palatinit® (Table 2).

DISCUSSION

Adapted rats were used to simulate a physiological status of regular dietary intake. Previous investigations on rat caecal and faecal flora revealed specific effects after adaptation to different sugars or sugar substitutes [7,21,22]. In vivo, the increase in bacterial number as well as the changes in catabolic enzyme activity and species composition indicate that selection is one of the adaptation mechanisms [23,24] leading to the increased ability of the flora to metabolize the respective substrate [14,21].

The influence of different metabolic history or diverse growth medium

composition on the shape of bacterial P–t curves [8,25] was confirmed when incubating the complex microflora in blank or supplemented growth medium (see dotted and solid lines in Fig. 1). Thereby, the total heat dissipated during the 48-h incubations reflects the extent of all catabolic processes [26]. As expected, degradation of additional substrate increased the heat output compared to that of the blanks (Fig. 2).

Since calculations and kinetic studies on microbial metabolism are difficult to quantitate when the accompanying heat evolution is complex [27], we estimated the energetic contribution due to the addition of sugars and sugar substitutes to the caecal flora cultures only if their influence exceeded the results obtained with the pure medium. The energy recovered in the form of heat (Table 1) did not differ significantly between the test substances; however, the lowest percentage recovered was with Polydextrose®. Since heat production depends on substrate catabolism, this trend can be related in tendency to its low degradation during the incubation period (highest substrate recovery, Table 1). The distinct difference in the degree of degradation between Polydextrose® and Palatinit® (Table 1) has also been demonstrated in vitro using digestive enzymes [6].

The specific heat productions (grand mean = 1.2 kJ/g substrate, Table 2) are within the limits of experimental error, in agreement with literature data on microbial anaerobic carbohydrate fermentation [11,28], and indicated that a fraction of about 7% of the combustible energy of the test substances is dissipated as heat during biodegradation. This value resembles closely the value of 6% mentioned by Walker [29] for carbohydrate fermentation by mixed rumen microflora, a comparable microbial community to that of the caecum [30]. Constant specific heat evolutions with various sugars as substrates have also been reported for the mixed bacterial ecosystem in soil [8].

It should be pointed out that, under physiological conditions, the heat generated in the lower part of the intestinal tract may not necessarily be an energy loss, because a caloric contribution to the thermal equilibrium of the host cannot be excluded [31].

Only for Polydextrose® and Palatinit® was a surplus of energy conversion into biomass calculated; no increase in relation to the blank yields was measured when glucose, sucrose or sorbitol were added to the medium (Table 1). This points to the limited capacity of a batch culture system in which high amounts of metabolic end products, e.g. fermentation acids, can accumulate. The resulting pH decrease may inhibit bacterial growth [32]. It has been demonstrated previously that low pH values can rapidly occur in vitro with these substrates, which are known to be easily available to intestinal bacteria [7,24,33]. Correspondingly, energy recovery in the form of acetate and lactate was highest in the glucose experiments (Table 1). Although large quantities of lactate are rarely found in vivo, significant colonic lactate production and pH lowering has been reported when greater amounts of readily fermentable carbohydrates are available [33].

The bacteriostatic decrease in pH did not occur in Polydextrose® and Palatinit® cultures [7], although substantial quantities of SCFA could be associated with the energy conversion of these substrates (Table 1). It might be assumed that simultaneous peptide catabolism of the basal medium occurred [34]. This would furnish alkaline fermentation products (i.e. ammonia), thus increasing the buffer capacity of the medium. This, however, occurs only when the rate of carbohydrate metabolism is not rapid [34] and implys that Polydextrose® and Palatinit® are degraded at a slower rate than glucose, sucrose or sorbitol. A lack of substantial pH decrease in a Palatinit® containing complex medium has also been reported for streptococcal cultures [35].

However, since the formation of microbial mass depends on substrate supply and affinity [36,37] it may be assumed that this energetic loss for the macro-organism differs between the alternative carbohydrates.

Specific energy conversion into absorbable metabolites indicates that the caloric salvage is twice as high for Polydextrose® (30% of the combustible energy) as for Palatinit® or sorbitol and is comparable to the value calculated for glucose (Table 2). Together, these results emphasize that the sugar substitutes tested cannot be regarded as a homogeneous group, considering their delayed caloric availability through fermentation. Thus, the proportion of utilizable energy available from the large intestine cannot be regarded as a constant fraction [15] and may depend on the nature and quantity of the alternative carbohydrate.

It is evident that the total recovery of gross energy of the added substrates (Table 1) cannot be complete under the given experimental conditions, because its conversion to gases and other common fermentation products [11] was not investigated. In addition, it must be considered that, in the range of realistic sugar intake, Polydextrose® and Palatinit® undergo marked small intestinal hydrolysis and digestion [38,39]. For instance, it cannot be excluded that, after small intestinal cleavage, mainly the hexitol parts of Palatinit® or some resistant fractions of Polydextrose® could enter the lower gut. For this reason further research is required to clarify the composition and nature of these carbohydrates entering the caecum after small intestinal passage.

Nevertheless, the present results indicate that this form of biological calorimetry in combination with a simple batch culture technique is a useful tool in evaluating the principles of energy conversion of nutrients by intestinal micro-organisms.

ACKNOWLEDGEMENTS

The authors thank Dr. L. Klingebiel for the analysis of the SCFA and Dr. Silvia Johnson for her help in preparing the manuscript.

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