# Recovery of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) in the small intestine of man

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The recovery of inulin, a naturally occurring $\beta(2 \rightarrow 1)$-fructan isolated from Jerusalem artichoke (*Helianthus tuberosus* L.), in the small intestine of man was studied in ileostomy subjects. The ileostomists were given a low-dietary-fibre diet based on white wheat bread and virtually free of inulin, and the same diet with the addition of 10 g and 30 g inulin product respectively, and the recovery and mean transit time (MTT) of inulin were estimated by tracking inulin in ileal effluent. The recovery of inulin was approximately 87% at both ingestion levels. MTT was 4·9 (SE 0·6) h at an intake of 10 g inulin product decreasing to 3·4 (SE 0·3) h at an intake of 30 g inulin product. A significant change in the fructose:glucose ratio of inulin from ingestion (4·1) to recovery in ileal effluent (4·5–4·7) and a lower recovery of the glucose residue than of the fructose residue of inulin indicate that the low-molecular-weight inulins are more sensitive to hydrolysis than the high-molecular-weight fragments. The loss of inulin during passage through the small intestine is presumably due to hydrolysis by either acids or enzymes and to microbial degradation by the microflora permanently colonizing the distal small intestine. The concentrations of lactic acid (LA) and short-chain fatty acids (SCFA) in frequently collected ileal effluents on the control day were approximately 6 mmol/l and approximately 55 mmol/l respectively. During periods with inulin ingestion the concentration of LA increased to 18–26 mmol/l ($P < 0.052$), while the concentration of SCFA ran converse and decreased to 18–32 mmol/l ($P < 0.023$). The osmotic loads (68 and 204 mosmol/l) associated with the ingestion of inulin product caused minor malabsorption of low-molecular-weight sugars.

Inulin: Ileostomy: Recovery: Man

Naturally-occurring plant $\beta(2 \rightarrow 1)$-fructans are found as osmoregulators and storage carbohydrates in a variety of vegetables including onions, garlic, asparagus and artichokes, in fruits such as bananas, and in cereals (Hirst, 1957; De Bruyn & Van Loo, 1991; Van Loo *et al.* 1995). Inulin is, for the purpose of this paper, defined as the polydisperse set of linear chain molecules made up of D-fructose residues linked to a terminal sucrose residue by means of $\beta(1-2')$ osidic bonds (De Leenheer & Hoebregs, 1994). The degree of polymerization (DP) is within the range 2 to 60 (Van Loo *et al.* 1995). The low-molecular-weight fractions of inulin (DP 2-20) are also known collectively as oligofructose (Van Loo *et al.* 1995).

Among the food plants, chicory (*Cichorium intybus*) and Jerusalem artichokes (*Helianthus tuberosus* L.) have proven to be rich sources of inulin and are well characterized as potential crops for commercial fructose production (Fleming & GrootWassink, 1979). These fructans have a slightly sweet taste which, however, decreases

with increasing DP. Inulin with a DP higher than 20 does not taste sweet any more (Archbold, 1940). This has led to the development of methods for isolation, purification and hydrolysis of higher-molecular-weight inulin from chicory by endoglycosidases.

The nutritional importance of inulin and other oligofructoses lies in their indigestibility. Only a small fraction is hydrolysed during their passage through the upper gastrointestinal tract (Yamashita *et al.* 1984; Stone-Dorshow & Levitt, 1987; Rumessen *et al.* 1990). Such carbohydrates have several potential nutritional advantages as low-energy dietary supplements, as sources of carbohydrates for diabetics and more generally as dietary fibre (Roberfroid, 1993). Studies have shown that inulin has a smaller glycaemic effect than fructose (Yamashita *et al.* 1984; Rumessen *et al.* 1990), and it has beneficial effects on microbial metabolism in the large intestine. Studies with rats and *in vitro* studies with bacteria isolated from the large intestine of man have indeed shown that inulin is almost completely metabolized in the large intestine where it serves as a selective growth substrate for bifidobacteria (Hidaka *et al.* 1986; Wang & Gibson, 1993). The nutritional energy value of inulin is consequently lower than that of sucrose, being in the range 4·2–6·3 kJ/g (Roberfroid *et al.* 1993). Therefore inulin isolated from Jerusalem artichoke or hydrolysed inulin from chicory are naturally-occurring carbohydrates which may be used by the food industry, not only as sugar substitutes but also as replacements for fat (Deis, 1994).

Two human studies have concluded that inulin is completely unabsorbed in the small intestine of man (Stone-Dorshow & Levitt, 1987; Rumessen *et al.* 1990). Both studies, however, were performed using the breath-$H_2$ test which is considered to be inaccurate by some (Cummings & Englyst, 1991). The ileostomy model provides a valuable alternative which may be used to study digestive physiology in man and it has often been used to study the small-intestinal excretion of nutrients (e.g. Langkilde *et al.* 1990; Schweizer *et al.* 1990; Cummings & Englyst, 1991). In the present investigation we measured the recovery of inulin with a DP higher than 2 in the small intestine of man using the ileostomy model.

### EXPERIMENTAL

#### Subjects

Seven subjects (six females and one male) with a median age of 38 (range 22–73) years participated in the study. All subjects had previously been proctocolectomized for ulcerative colitis (six) or familial polyposis coli (one). All were in good health and had well-established ileostomies without signs of small-bowel obstruction or ileostomy dysfunction. No resection of the small intestine or stomach had been performed and no antibiotics had been given within the last week. All had firm to thick ileostomic contents with a maximum of 1 litre/d. The study protocol was approved by the Aarhus County Medical Ethics Committee.

#### Protocol

The subjects were admitted to the hospital for 3 d. On the first two successive days we studied the recovery of 10 g and 30 g respectively of the inulin product, while the control day followed a period of normal food intake. Since the inulin was recovered in ileal effluents in less than 11 h, the authors do not believe that there was any carry-over effect that influenced the interpretation of the results.

#### Diet

The study was divided into three 24 h periods starting at 08.00 hours each day. The basal diet consisted of white wheat bread, butter, cheese, egg, ham, omelette, cured saddle of pork, pickled meat, fresh slices of meat, milk low in fat, water, tea, coffee and a glass of

Table 1. *Carbohydrate composition (g/kg) of the inulin product ingested*

(Mean values with their standard errors for five determinations)

|  | Mean | SE |
|---|---|---|
| Total carbohydrate | 919·0 | 0·04 |
| Glucose | 8·4 | 0·3 |
| Fructose | 18·7 | 0·3 |
| Sucrose | 184·3 | 1·0 |
| Inulin | 707·6 | 3·3 |
| Fructose:Glucose ratio of inulin | 4·10 | 0·046 |

white wine with the dinner. The menus were the same on all three study days and provided: energy 8·8 MJ, protein 105 g, fat 101 g and carbohydrate 159 g. The day before the investigation the subject had her or his last meal at 20.00 hours, after which she or he was only allowed to consume water, tea and coffee. The main meals were served at 08.00, 12.00 and 18.00 hours.

The inulin product used in the present study was purified from Jerusalem artichoke (Danisco A/S, Danish Sugar Cooperation, Nakskov, Denmark) and had a carbohydrate composition as shown in Table 1. The chain-length distribution (%) was: DP3 12·9, DP4 12·9, DP5 11·2, DP6 9·8, DP7 8·2, DP8 6·2, DP9 4·5, DP10 2·8 and DP > 10 31·5.

The inulin product (10 g or 30 g) was dissolved in 200 ml water and taken at 10.00 hours. The osmolarity of the load was 68 mosmol/l at the low intake level (10 g inulin product) and 204 mosmol/l at the high intake level (30 g inulin product). The rationale of using this testing scheme was that the isolated inulin product typically will be incorporated into products consumed at intervals between main meals.

### Effluent

Ileostomy effluent was collected by the subjects when they emptied or changed their bags every 2 h from 08.00 hours to 20.00 hours, again when they retired at 23.00 hours and the next morning at 08.00 hours. Each sample of effluent was frozen immediately after collection, stored at −18° and mixed thoroughly before samples were taken for analysis.

### Chemical analyses

The dry matter (DM) content of ileal effluents was determined by freeze-drying and that of the inulin product by drying to constant weight at 70° in a vacuum oven. All analyses of ileal effluent were made in duplicate while the carbohydrate composition of the inulin product was determined in five replicates. The low-molecular-weight (LMW) sugars (glucose, fructose and sucrose) and inulin (DP > 2) were determined in individual collections of freeze-dried materials, while ash, N, starch and non-starch polysaccharides (NSP) were determined in pooled freeze-dried samples. Organic acids were analysed either in pooled wet or freeze-dried materials.

Protein (N × 6·25) was determined by the Kjeldahl method using a Kjell-Foss 16200 autoanalyser and ash was measured as described by the Association of Official Analytical Chemists (1990). Starch was analysed by a modification of the enzymic method of Bach Knudsen *et al.* (1987). Starch was gelatinized and quantitatively removed by incubation (100°, 60 min; 60°, 2 h) with a thermostable α-amylase (*EC* 3.2.1.1; Termamyl®, Novo Nordisk A/S, Copenhagen, Denmark) and a β-glucanase-free amyloglucosidase (*EC* 3.2.1.3; Cat No. 1060 074, Boehringer Mannheim GmbH, Mannheim, Germany)

and the resulting glucose monomers were quantified with a glucose oxidase reagent
($EC$ 1.1.3.4; Cat No. 124001, Boehringer Mannheim GmbH). To correct for coloured
substances in ileal effluent a blank was carried through the whole procedure. Total NSP and
their constituent sugars in ileal effluent were determined as alditol acetates by GLC for
neutral sugars using a modification of the Uppsala (Theander & Åman, 1979; Theander &
Westerlund, 1986) and the Englyst (Englyst $et$ $al.$ 1982) procedures (Bach Knudsen $et$ $al.$
1993), and by a colorimetric method for uronic acids (Scott, 1979).

LMW sugars and inulin in the inulin product and ileal materials were analysed as
follows. Duplicate samples of approximately 400 mg ileal effluent were weighed into 50 ml
centrifuge tubes with screw caps. Water (25 ml) was added and the samples extracted for
70 min at 65°. During extraction the centrifuge tubes were mixed (vortex mixer) at least
three times. The tubes were centrifuged (2200 $g$, 20 min) and a portion of the supernatant
fraction was used directly for free glucose and fructose determinations, a portion was
hydrolysed with 0·037 M-$H_2SO_4$ (80°, 70 min) and a portion was used for HPLC
determination. Glucose and fructose in the water extract before and after acid hydrolysis
were quantified in a coupled enzymic reaction with $NADP^+$. The reaction between glucose
and $NADP^+$ is catalysed by the enzymes hexokinase ($EC$ 2.7.1.1; Boehringer Mannheim
GmbH) and glucose-6-phosphate dehydrogenase ($EC$ 1.1.1.49; Boehringer Mannheim
GmbH). Fructose-6-phosphate is first converted to glucose-6-phosphate by phospho-
glucose isomerase ($EC$ 5.3.1.9; Boehringer Mannheim GmbH) and then quantified as
above. The amount of NADPH formed during oxidation of $NADP^+$ was stoichiometrically
related to the amount of glucose and fructose in the sample. The portion taken for HPLC
determination was mixed (9:1) with internal standard (IS; arabinose, 11·1 mg/ml), to give
a final IS concentration of 1 mg/ml. A 2 ml portion was filtered through a Bond Elute $C_{18}$
cartridge (Analytichem International, Harbor City, CA, USA) prewetted with 2 ml
methanol and 5 ml deionized water; the first 1·5 ml eluate was collected and further filtered
through a 0·22 $\mu$m PTFE filter (Minisart NMLPF, Sartorius AG, Göttingen, Germany)
and 20 $\mu$l was used for HPLC determination. The HPLC system used consisted of a Model
510 solvent delivery pump, Model 410 refractive index detector, temperature control
module (Waters Chromatography Division, Milford, MA, USA), Model 7126 injector
valve (Rheodyne Inc. Catati, CA, USA), Model LCI-100 recording integrator with built-
in printer/plotter (Perkin-Elmer Inc., Norwalk, CT, USA) and a Shodex Ionpak KS-901
(8 mm × 300 mm) resin-based column in the sodium form (Showa Denko K.K., Tokyo,
Japan). Water was used as mobile phase, the flow rate was 0·6 ml/min and the column
temperature was kept constant at 85°.

LMW sugars in the inulin product and ileal effluent were calculated as:

$$\text{LMW sugars} = (\text{Glc}_F + \text{Fru}_F + \text{Sucrose}_{HPLC}) \qquad (1)$$

and inulin as:

$$\text{inulin} = (\text{Glc}_A + \text{Fru}_A - \text{Glc}_F - \text{Fru}_F - \text{Sucrose}_{HPLC}) \times 0.93 \qquad (2)$$

where $\text{Glc}_A$ and $\text{Fru}_A$ are the total amounts of glucose and fructose estimated after acid
hydrolysis, $\text{Glc}_F$ and $\text{Fru}_F$ are the amounts of the two monosaccharides found in the water
extract, $\text{Sucrose}_{HPLC}$ is the amount of sucrose estimated by HPLC and 0·93 is the
conversion factor from monosaccharides to oligosaccharides. The molecular weight
distribution was determined by GLC according to the method of Quemener $et$ $al.$ (1994).

Total short-chain fatty acids (SCFA) were determined on wet or freeze-dried materials
using a modification of the method of Fussell & McCalley (1987). Duplicate samples of
either 1·5 g wet ileal digesta or 0·5 g freeze-dried digesta were weighed into 50 ml centrifuge
tubes with screw caps. Oxalic acid (0·3 M) was added to the samples; approximately 6 ml
(four times the wet weight) to wet samples and 10 ml (twenty times the dry weight) to dry

samples. The centrifuge tubes were capped, shaken mechanically for 1 h and then centrifuged (15 000 $g$, 10 min). A 5 ml portion was mixed with 0·5 ml pivalic acid (IS; 2·5 g/l in 0·3 M-oxalic acid), centrifuged (15000 $g$, 10 min) and filtered through a 0·45 $\mu$m PTFE filter. Separation of the individual SCFA was performed by GLC on a Perkin Elmer 8310 gas chromatograph (Perkin Elmer Inc. USA) equipped with flame-ionization detector. The column was a glass coil (2 m × 3 mm i.d.) packed with Carbopack B-DA 80-120 mesh (Supelco Inc., PA, USA). Column temperature was kept constant at 175° and the injector and detector temperatures were maintained at 200°. $N_2$ with a flow rate of 24 ml/min was used as carrier gas. Total lactic acid (LA) was determined in wet or freeze-dried samples by means of specific enzymes in a coupled enzymic reaction with $NAD^+$. Duplicate samples of either 5 g wet ileal digesta or 0·5 g freeze-dried digesta were weighed into 50 ml centrifuge tubes with screw caps. To the wet ileal samples was added 25 ml sodium azide (2 g/l) and to the dry samples 20 ml sodium azide (2 g/l). The centrifuge tubes were capped, shaken mechanically for 15 min and centrifuged (4000 $g$, 5 min). Quantification of LA in the supernatant fraction was done in a coupled enzymic reaction with $NAD^+$ after decolorizing the supernatant fraction by transferring it to a test-tube containing 1 g polyvinyl-polypyrrolidone (Sigma Chemicals Company, St. Louis, MO, USA), precipitating coloured substances for 15 min and finally centrifuging (4000 $g$, 5 min). The reaction between L-LA, D-LA and $NAD^+$ is catalysed by the enzymes L-lactate dehydrogenase ($EC$ 1.1.1.27; Boehringer Mannheim GmbH) and D(−)-lactate dehydrogenase ($EC$ 1.1.1.28; Boehringer Mannheim GmbH). The amount of NADH formed during the oxidation of $NAD^+$ was stoichiometrically related to the amount of LA in the sample.

### Calculations and statistical analysis

Each sample of effluent from the 2 d ingestion of inulin product was analysed for LMW sugars and inulin. Ash, starch, N, NSP, LA and SCFA analyses were done on samples pooled over the period where inulin was detected in ileal effluents (mostly 10.00–20.00 hours; the range for the lower boundary was 10.00–12.00 hours and for the higher boundary 18.00–22.00 hours) and from periods where there was none or only trace levels of inulin in ileal effluent (08.00–10.00 hours and 20.00–08.00 hours). The last pooled samples represent periods with less frequent emptying and sampling of the ileal bags. The samples from the control day were pooled according to an average of those of the 2 d with inulin ingestion and the samples analysed for ash, N, starch, NSP, LMW sugars, inulin, LA and SCFA.

The mean transit time (MTT) of inulin was calculated as:

$$\text{MTT (h)} = \frac{\sum_{0}^{N} (\text{amount of inulin in sample}) \times t}{\text{Total inulin recovered}}, \qquad (3)$$

where $t$ is the time interval between the test meal and recovery of inulin in effluent from sample 0 to $N$.

The results from the study were analysed as a randomized block experiment as described by Snedecor & Cochran (1973):

$$X_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}, \qquad (4)$$

where $X_{ij}$ is the dependent variable (e.g. recovery), $\mu$ is the overall mean, $\alpha_i$ is the effect of levels of ingestion of inulin, $\beta_j$ is the block effect (individuals) and $\epsilon_{ij}$ is a normally distributed random variable. Significant differences between treatment means were identified by Scheffe's S test (Snedecor & Cochran, 1973). All statistical calculations were done using a SuperAnova package (Abacus Concepts, Berkeley, CA, USA).

## RESULTS

The inulin product had a total carbohydrate content of 919·0 g/kg with a distribution of (g/kg): glucose 8·4, fructose 18·7, sucrose 184·3 and inulin 707·6 (Table 1). The fructose:glucose ratio of inulin was 4·1. Recoveries (%) of sucrose and inulin in spiked samples (40 mg inulin product) were: sucrose 102 (SE 3·4), and inulin 100 (SE 2·0). On the basis of this analysis the intake of inulin (DP > 2) was estimated to be 7·1 g at an intake of 10 g inulin product and 21·2 g inulin at an intake of 30 g inulin product. The additional intakes of LMW sugars at the two levels of inulin product ingestion were 2·1 g (10 g inulin product) and 6·3 g (30 g inulin product).

The average composition of the 24 h ileal effluent from the control day and after ingestion of 10 g and 30 g of inulin product is shown in Table 2. Total effluent increased from 413 (SE 85) g on the control day to 493 (SE 72) g and 633 (SE 93) g at the intakes of 10 g and 30 g inulin product respectively; the dry solid increased from 41·7 (SE 5·8) g to 56·1 (SE 4·9) g and 69·4 (SE 6·7) g with the same treatments. The increase in dry solid was primarily caused by a higher excretion of carbohydrate in effluent whereas ash and N remained almost constant. The total amount of carbohydrate in ileal effluent was 10·2 g representing 331 g/kg dry solid on the control day, increasing to 18·1 g (331 g/kg) and 31·0 g (464 g/kg) at intakes of 10 g and 30 g inulin product respectively.

The increase in the carbohydrate content of dry solid in response to inulin ingestion was primarily caused by a higher excretion of inulin in effluent (Table 3). The basal diet provided an almost negligible amount of inulin (0·2 g) whereas it increased to 6·1 (SE 0·3) g and 18·4 (SE 0·6) g at the low and high intakes of inulin product respectively. LMW sugars also increased ($P < 0.001$) in response to inulin ingestion from a level of 0·7 (SE 0·1) g on the control day to 1·1 (SE 0·1) g and 1·6 (SE 0·1) g at the low and high levels of inulin ingestion. In contrast there was no significant effect of the increased inulin ingestion on any of the polysaccharide fractions. The excretion of starch in ileal effluent was 1·0–1·2 g ($P < 0.66$) and the excretion of NSP 8·2–9·9 g ($P < 0.41$).

The passage of inulin through the gut was monitored by measuring the output of inulin in each 2 h collection after intakes of 10 g and 30 g inulin product respectively. As shown in Fig. 1, the average output of inulin in ileal effluent of the seven subjects after inulin ingestion increased rapidly and peaked 3 h after dosing at both dosing levels. At peak output levels (3 h post dosing) inulin represented approximately 230 g/kg ileal dry solid with an intake of 10 g inulin product and approximately 550 g/kg dry solid at an intake of 30 g inulin product. After 11 h the effluent was almost free of inulin.

The two levels of inulin ingestion did not affect the recovery of inulin in ileal effluent which was estimated to be 86·4 (SE 4·4) % at the low level of inulin ingestion and 87·0 (SE 3·3) % ($P < 0.85$) at the high level of ingestion (Table 4). The fructose:glucose ratio of the recovered inulin in ileal effluent was 4·7 (SE 0·1) at an intake of 10 g inulin product and 4·5 (SE 0·1) at an intake of 30 g inulin product ($P < 0.24$). Concurrently the recovery of the inulin glucose residue was 77·3–80·2 %, which was significantly lower ($P < 0.001$) than that of the inulin fructose residue which was about 88·5 %. MTT was significantly lower at the high dosing level and decreased from 4·9 h at the intake of 10 g inulin product to 3·4 h ($P < 0.005$) at the intake of 30 g inulin product. The correlation between the MTT estimated at 10 g and 30 g inulin ingested was high ($r$ 0·79, $P < 0.001$). The recovery of inulin in ileal effluent, however, was not related to the MTT as the correlation between MTT and the recovery of inulin was only $-0.27$ ($P < 0.37$) (Fig. 2).

To study the recovery of organic acids in freeze-dried ileal samples, wet and freeze-dried materials from the control day were analysed for LA and SCFA. Freeze-drying did not result in any significant loss of either LA or SCFA as the concentration of LA in the periods

Table 2. *Composition of the ileal effluent from ileostomy subjects consuming a control diet, or the same diet supplemented with 10 or 30 g inulin product\**

(Mean values with their standard errors for seven ileostomy subjects)

| | Control day | | Inulin product ingested (g) | | | | P value for dietary treatment |
| | | | 10 | | 30 | | |
| | Mean | SE | Mean | SE | Mean | SE | |
|---|---|---|---|---|---|---|---|
| Total effluent (g/d) | 413 | 85 | 493 | 72 | 633 | 93 | 0·13 |
| Total solids (g/d) | 41·7[b] | 5·8 | 56·1[a, b] | 4·9 | 69·4[a] | 6·7 | 0·010 |
| Ash (g/d) | 6·9 | 1·1 | 6·3 | 0·7 | 7·3 | 1·1 | 0·74 |
| Organic matter (g/d) | 34·8[b] | 4·8 | 49·3[a, b] | 4·3 | 61·2[a] | 5·6 | 0·004 |
| Total carbohydrates (g/d) | 10·2[c] | 1·1 | 18·1[b] | 0·9 | 31·0[a] | 1·4 | 0·001 |
| Total carbohydrates (g/kg solids) | 250[c] | 16 | 331[b] | 17 | 464[a] | 31 | 0·001 |
| Nitrogen (g/d) | 2·1 | 0·3 | 2·5 | 0·3 | 2·1 | 0·3 | 0·51 |
| N × 6·25 (g/d) | 12·9 | 2·1 | 15·5 | 1·9 | 13·3 | 1·6 | 0·51 |
| Nitrogen × 6·25 (g/kg solids) | 308[a] | 11 | 272[b] | 11 | 190[c] | 9 | 0·001 |

[a, b, c] Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

\* For details of inulin product and procedures, see Table 1 and pp. 102–105.

Table 3. *Carbohydrate composition (g/d) of the ileal effluent from ileostomy subjects consuming a control diet, or the same diet supplemented with 10 or 30 g inulin product\**

(Mean values with their standard errors for seven ileostomy subjects)

| | Control day | | Inulin product ingested (g) | | | | P value for dietary treatment |
| | | | 10 | | 30 | | |
| | Mean | SE | Mean | SE | Mean | SE | |
|---|---|---|---|---|---|---|---|
| LMW sugars | 0·7[c] | 0·1 | 1·1[b] | 0·1 | 1·6[a] | 0·1 | 0·001 |
| Inulin | 0·2[c] | 0·1 | 6·1[b] | 0·3 | 18·4[a] | 0·6 | 0·001 |
| Starch | 1·0 | 0·1 | 1·2 | 0·2 | 1·2 | 0·2 | 0·66 |
| Total NSP | 8·2 | 0·9 | 9·8 | 0·8 | 9·9 | 1·1 | 0·41 |
| Rhamnose | 0·1 | 0·01 | 0·1 | 0·02 | 0·1 | 0·02 | 0·80 |
| Fucose | 0·5 | 0·05 | 0·5 | 0·05 | 0·5 | 0·05 | 0·49 |
| Arabinose | 1·4 | 0·2 | 1·7 | 0·2 | 1·6 | 0·2 | 0·52 |
| Xylose | 1·9 | 0·3 | 2·4 | 0·2 | 2·4 | 0·3 | 0·36 |
| Mannose | 0·6 | 0·1 | 0·8 | 0·1 | 0·9 | 0·1 | 0·16 |
| Galactose | 1·3 | 0·2 | 1·4 | 0·2 | 1·5 | 0·2 | 0·57 |
| Glucose | 2·3 | 0·2 | 2·6 | 0·2 | 2·7 | 0·3 | 0·41 |
| Uronic acids | 0·2 | 0·03 | 0·2 | 0·02 | 0·2 | 0·03 | 0·99 |

LMW sugars, low-molecular-weight sugars (glucose, fructose and sucrose); NSP, non-starch polysaccharides.

[a, b, c] Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

\* For details of inulin product and procedures, see Table 1 and pp. 102–105.

with frequent collections was 7·3 (SE 0·6) mmol/l in wet samples and 5·9 (SE 0·9) mmol/l in freeze-dried samples ($P < 0.42$) and the concentration of SCFA was 51·7 (SE 13·2) mmol/l in wet samples and 55·4 (SE 12·0) mmol/l in freeze-dried samples ($P < 0.71$) (Table 5). In periods with less frequent collections the concentration of LA estimated in wet samples was 10·4 (SE 1·1) mmol/l and in freeze-dried samples 10·1 (SE 2·3) mmol/l ($P < 0.54$), and that of SCFA in wet samples was 83·9 (SE 16·3) mmol/l compared with 83·3 (SE 11·3) mmol/l in
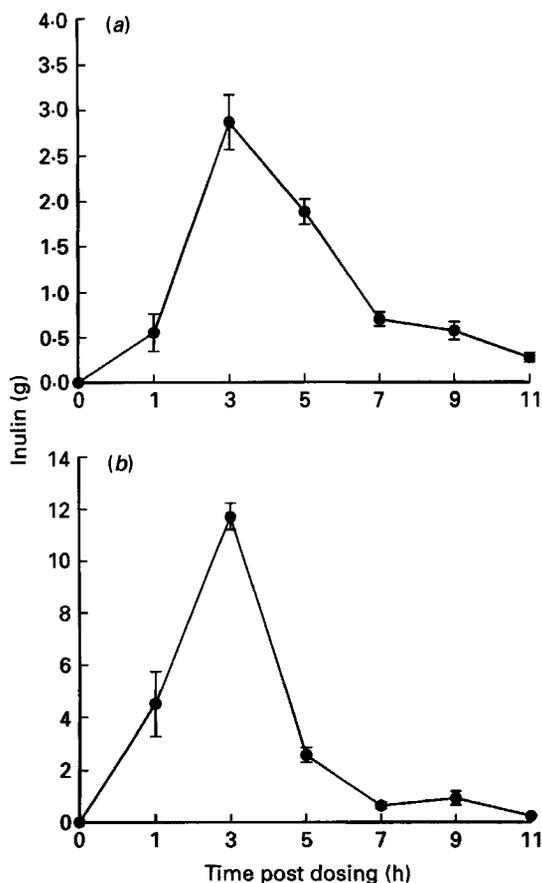
Fig. 1. Recovery of inulin (g) in ileal effluent following ingestion of (a) 10 g and (b) 30 g inulin product on successive days. The values are means of seven ileostomy subjects with their standard errors represented by vertical bars.

Table 4. *Excretion of inulin in ileal effluent, and mean transit time (MTT) of inulin in ileostomy subjects consuming a control diet, or the same diet supplemented with 10 or 30 g inulin product**

(Mean values with their standard errors for seven ileostomy subjects)

| | Control day | | Inulin product ingested (g) | | | | $P$ value for dietary treatment |
| | | | 10 | | 30 | | |
| | Mean | SE | Mean | SE | Mean | SE | |
|---|---|---|---|---|---|---|---|
| Inulin (g/d) | 0·2[c] | 0·1 | 6·1[b] | 0·3 | 18·4[a] | 0·6 | 0·001 |
| Fructose:Glucose ratio of inulin | — | | 4·7 | 0·1 | 4·5 | 0·1 | 0·24 |
| Inulin recovery (%) | — | | 86·4 | 4·4 | 87·0 | 3·1 | 0·85 |
| Inulin-glucose recovery (%) | — | | 77·3 | 4·7 | 80·2 | 2·5 | 0·52 |
| Inulin-fructose recovery (%) | — | | 88·5 | 4·3 | 88·4 | 3·3 | 0·97 |
| MTT inulin (h) | — | | 4·9[a] | 0·6 | 3·4[b] | 0·3 | 0·005 |

[a,b,c] Mean values within a row with different superscript letters were significantly different ($P < 0.05$).
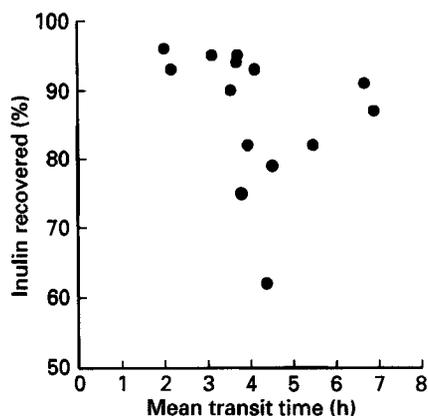* For details of inulin product and procedures, see Table 1 and pp. 102–105.

Fig. 2. Correlation between the recovery of inulin (as a percentage of inulin intake) in ileal effluent, and the mean transit time of inulin through the small intestine. $r -0.27$, $P < 0.37$.

Table 5. *Lactic acids (LA) and short-chain fatty acids (SCFA) in pooled wet and freeze-dried ileal effluent from ileostomy subjects fed on a low-dietary-fibre basal diet*\*

(Mean values with their standard errors for seven ileostomy subjects)

| Period† | LA (mmol/l) | | | | SCFA (mmol/l) | | | |
|---|---|---|---|---|---|---|---|---|
| | Wet | Freeze-dried | SE | *P* value | Wet | Freeze-dried | SE | *P* value |
| 1 | 7·3 | 5·9 | 0·7 | 0·42 | 51·7 | 55·4 | 13·4 | 0·71 |
| 2 | 10·4 | 10·1 | 1·2 | 0·54 | 83·9 | 83·3 | 15·0 | 0·91 |

\* For details of diet and procedures, see pp. 102–105.

† Period 1, pooled effluent from 10.00–20.00 hours; Period 2, pooled effluent from 08.00–10.00 hours and 20.00–08.00 hours.

freeze-dried samples ($P < 0.91$). The overall correlation between LA and SCFA estimated on wet or freeze-dried materials respectively, was 0·95 ($P < 0.001$) for LA and 0·99 ($P < 0.001$) for SCFA.

The concentration of LA in the ileal effluents collected frequently was 5·9 (SE 0·9) mmol/l on the control day. This level increased to 25·8 (SE 7·3) mmol/l at the low ingestion level of inulin and to 18·5 (SE 6·8) mmol/l at the high ingestion level of inulin ($P < 0.052$; Table 6). The opposite was the case with the concentration of SCFA, which decreased from 55·4 (SE 12·0) mmol/l in the effluent from the control day to a level of 31·9 (SE 8·1) mmol/l at an ingestion of 10 g inulin product and 18·3 (SE 3·6) mmol/l at an ingestion of 30 g inulin product ($P < 0.023$). The concentration of SCFA in ileal effluent was in general higher during periods with less frequent collections with values in the range 52·7–83·3 mmol/l. The same was the case with LA on the control day, while the opposite was the case on days with inulin ingestion. The excretion of LA in ileal effluent over 24 h increased from 2·7 (SE 0·4) mmol/d on the control day to 7·0 (SE 2·0) and 8·8 (SE 1·9) mmol/d with ingestion levels of 10 g and 30 g inulin product respectively. The reverse was seen with SCFA, which decreased from 23·6 (SE 3·8) mmol/d on the control day to 15·5 (SE 2·3) and 15·8 (SE 2·8) mmol/d at 10 g and 30 g inulin product ingested respectively.

Acetic acid was the predominant SCFA in ileal effluent accounting for 0·88–0·90 of the acids, while propionic acid (0·02–0·05) and butyric acid (0·05–0·06) were minor components.

Table 6. *Organic acid content of ileal effluent from ileostomy subjects consuming a control diet, or the same diet supplemented with 10 or 30 g inulin product**

(Mean values with their standard errors for seven ileostomy subjects)

| | Control day | | Inulin product ingested (g) | | | | P value for dietary treatment |
| | | | 10 | | 30 | | |
| | Mean | SE | Mean | SE | Mean | SE | |
|---|---|---|---|---|---|---|---|
| LA (mmol/l)/Period 1 | 5·9 | 0·9 | 25·8 | 7·3 | 18·5 | 6·8 | 0·052 |
| LA (mmol/l)/Period 2 | 10·1 | 2·3 | 9·4 | 4·0 | 15·1 | 4·9 | 0·43 |
| SCFA (mmol/l)/Period 1 | 55·4ᵃ | 12·0 | 31·9ᵃ·ᵇ | 8·1 | 18·3ᵇ | 3·6 | 0·023 |
| SCFA (mmol/l)/Period 2 | 83·3 | 11·4 | 52·7 | 10·0 | 59·0 | 16·6 | 0·26 |
| SCFA (proportion of total)† | | | | | | | |
| Acetic acid | 0·90 | 0·02 | 0·89 | 0·02 | 0·88 | 0·02 | 0·59 |
| Propionic acid | 0·02 | 0·01 | 0·05 | 0·02 | 0·05 | 0·02 | 0·08 |
| Butyric acid | 0·06 | 0·002 | 0·05 | 0·02 | 0·06 | 0·02 | 0·65 |
| LA (mmol/d) | 2·7ᵇ | 0·4 | 7·0ᵃ·ᵇ | 2·0 | 8·8ᵃ | 1·9 | 0·021 |
| SCFA (mmol/d) | 23·6 | 3·8 | 15·5 | 2·3 | 15·8 | 2·8 | 0·162 |

Period 1, pooled effluent from 10.00–20.00 hours; Period 2, pooled effluent from 08.00–10.00 hours and 20.00–08.00 hours. LA, lactic acid; SCFA short-chain fatty acids.

[a,b,c] Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

* For details of diet and procedures, see pp. 102–105.

† Pooled effluents from Periods 1 and 2.

## DISCUSSION

The present study, performed with ileostomists, demonstrates that inulin is practically indigestible in the small intestine of man. The recovery of inulin in ileal effluent was approximately 87 % at both the low (10 g inulin product) and the high (30 g inulin product) intake levels, thus confirming *in vitro* studies using human saliva, rat pancreas homogenates, and rat and human small-intestinal mucosa which demonstrated that inulin is hydrolysed only marginally by mammalian intestinal enzymes (Oku *et al.* 1984; Nilsson *et al.* 1988). The results of the present study are also in good agreement with human studies using the breath $H_2$ test, which have concluded that inulin is virtually completely unabsorbed in the upper part of the small intestine (Stone-Dorshow & Levitt, 1987; Rumessen *et al.* 1990).

The small, though significant, loss of inulin during passage through the small intestine may be due to hydrolysis by either acid or enzymes or to microbial degradation by the microflora permanently colonizing the distal small intestine. The significant change in the fructose:glucose ratio of inulin from ingestion (fructose:glucose 4·1) to recovery in the ileal effluent (4·5–4·7) strongly indicates that low-molecular-weight components of inulin are more sensitive to hydrolysis than the high-DP fragments. In experiments with Neosugar® (a fructo-oligosaccharide with two to four fructosyl residues linked to a terminal glucose; Meiji Seika Kaisha Ltd., Kanto, Japan; Oku *et al.* 1984) it has been found that the hydrolysis by rat intestinal mucosa occurs at a rate < 0·5% of that of sucrose. The likely cause is that the enzyme affinity for the low-molecular-weight fructo-oligosaccharides, when compared with that for sucrose, is affected by the higher molecular size. It is therefore more likely that the hydrolysis of the low-DP inulin is due to gastric juice. In an *in vitro* study with isolated fructan fractions from wheat, Nilsson *et al.* (1988) found a hydrolysis of 15–16% per h for fructans with DP of 3 and 4 at a pH of 1 declining to about 5% per h at a pH of 1·5. These hydrolyses of cereal fructans were similar to that of sucrose but higher than that of a cereal fructan with a DP of 9. However, it should be mentioned that

the pH used in these *in vitro* experiments is presumably lower than *in vivo* when the pH is buffered by the bolus.

Potentially inulin could also be fermented by the microbial population colonizing the distal part of the small intestine. A major difference between the normal ileum and that of the ileostomists is indeed its microbial population, which is greater in the ileostomists ($10^7$–$10^8$ bacteria/g) compared with the normal ileum ($10^5$–$10^8$ bacteria/g; Finegold *et al.* 1970; Drasar & Hill, 1974). The outcome of this colonization is the microbial breakdown of carbohydrates and the formation of LA and SCFA. In the present study we found a concentration of LA of approximately 6 mmol/l and of SCFA of approximately 55 mmol/l on the control day with a tendency towards a lower concentration of the acids in periods with frequent collections (10.00–20.00 hours) compared with less frequent collections (mostly night). The levels of SCFA found in the present study are far higher than previously reported by Englyst & Cummings (1986) who failed to reveal SCFA at > 3–9 mmol/l concentrations in any ileostomy subjects, but lower than in the caecum where fermentation is active and the concentration of SCFA is well in excess of 100 mmol/l (Cummings *et al.* 1987). In spite of the higher level of organic acids in ileal effluents compared with previous findings by Englyst & Cummings (1986) there was no indication of either a higher concentration of organic acids or an increased excretion in the presence of inulin. This is presumably due to the fact that the capacity of the microflora for metabolizing carbohydrates into organic acids is met even with the level of carbohydrate present on the control day. However, the results seem to indicate that the type of substrate utilized by the flora may have been altered from the control day to periods with inulin ingestion resulting in a change in the nature of the fermentation products formed. The losses of inulin from ingestion to recovery in the ileum amounted to 1·1 and 3·4 g/d at the low and high ingestion levels of inulin respectively, which can be compared with an increased excretion of 2·2 and 2·8 g/d of LMW sugars, starch and NSP, at the two ingestion levels of inulin respectively. Thus, approximately the same amount of carbohydrate was fermented (2–3 g/d as calculated from the excretion of LA and SCFA) but the nature of the fermentation products changed (more LA at the expense of SCFA), perhaps because of changes in substrate: i.e. in the presence of inulin, bacteria utilize it in preference to starch and NSP.

Neither the concentration of organic acids nor the total excretion of organic acids in ileal effluent is therefore regulated by dietary composition but is a result of the permanent flora colonizing this part of the gastrointestinal tract. The molar composition of SCFA in ileal effluent is quite constant and typical for materials collected from the small intestine of man and single-stomached animals like the pig (Cummings *et al.* 1987; Bach Knudsen *et al.* 1993). For comparison it can be mentioned that while acetic acid typically accounts for 80–90 % of the pool of SCFA in the distal small intestine of man and pigs, acetic acid decreases to 50–60 % of SCFA in the caecum (Cummings *et al.* 1987; Bach Knudsen *et al.* 1993).

The transit of inulin through the small intestine was rapid, with a MTT of 4·9 (SE 0·6) h at an ingestion of 10 g inulin product and 3·4 (SE 0·3) h at an ingestion of 30 g inulin product. In contrast to what was found for resistant starch type 3 (retrograded amylose), where the correlation between MTT and the recovery was found to be highly significant ($r$ −0·6), the correlation between MTT and the recovery of inulin was only −0·27. The transit of inulin through the small intestine is faster than that of NSP in mixed diets where MTT of potato, banana and cereal were estimated to be 6–8 h (Englyst & Cummings, 1985, 1986, 1987). The MTT of inulin in the present study, however, was longer than that found using the breath $H_2$ test where the orocaecal transit time was found to be about 3 h for 10 g inulin product compared with about 7 h for 50 g wheat starch when the two carbohydrate sources were provided as the sole source of carbohydrate (Rumessen *et al.* 1990).

Although the osmotic load (68 and 204 mosmol/l) was within the safe osmotic range of the small intestine, ingestion of inulin resulted in a minor malabsorption of LMW sugars. In response to the increased intake of inulin there was an associated increase in LMW sugars in ileal effluent which increased from 0·7 g/d on the control day to 1·1 g/d with an intake of 10 g inulin product, and further to 1·6 g/d with an intake of 30 g inulin product. The increase in LMW sugars of ileal effluent is minor relative to the other carbohydrates but the difference was highly significant and in striking contrast to that found for the polysaccharides. Neither the output of starch nor total NSP and its residues were influenced by the ingestion of inulin. Furthermore, in spite of the fact that the inulin was not given together with the breakfast meal the result is consistent with an earlier finding showing that inulin, in contrast to some dietary fibres, does not influence wheat starch absorption when inulin and wheat starch are provided together (Rumessen et al. 1990).

In terms of indigestibility inulin shares many features with dietary fibre (Roberfroid, 1993). The recovery is within the same order as found for soluble fibres like pectin (Sandberg et al. 1983), while it is slightly lower than that reported for cereal foods by Englyst & Cummings (1985) and potato by Schweizer et al. (1990). On the basis of its indigestibility it would therefore be natural to consider inulin or oligofructose and other non-digestible oligosaccharides in the same way as dietary fibre components. These substances all provide energy to the microbial population that inhabits the human caecum and colon. In addition, inulin may have some specific beneficial properties for the microbial metabolism in the large intestine (Hidaka et al. 1986; Roberfroid et al. 1993). Studies have shown that inulin serves as a selective growth substrate for bifidobacteria.

In conclusion, the present study shows that inulin, isolated from Jerusalem artichoke, is virtually indigestible in the small intestine of man. In terms of indigestibility the inulin, like other oligofructoses, has features similar to those of dietary fibre.

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