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Dose–response effects of raw potato starch on small-intestinal escape, large-bowel fermentation and gut transit time in the rat

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This study was designed to quantify starch digestion within the small and large bowels separately when raw potato starch (RPS) was included at 0–240 g/kg in diets fed to growing male Wistar rats. RPS was incorporated in the diets at the expense of maize starch which was expected to be almost completely digested in the small bowel. The digestibility of the maize starch was 0·99 but only 0·28 of the RPS was digested before the terminal ileum so that with increasing intakes of RPS there was a progressive increase in starch supply to the large bowel (LB). Of this starch 0·77, 0·72 and 0·73 was fermented in the large bowel when RPS constituted 80, 160 and 240 g/kg diet respectively. With increasing RPS intake, there was a curvilinear response in molar proportion of butyrate in caecal contents with a maximum value at about 80 g RPS/kg diet. The molar proportion of acetate increased linearly, that of propionate was unchanged, whilst proportions of the minor short-chain fatty acids all declined markedly with increasing RPS intake. The novel marker *Bacillus stearothermophilus* spores (BSS) was compared with CrEDTA in estimation of whole-gut mean transit time (MTT) when given together in a single test meal. Whilst estimates of MTT for the two markers were strongly correlated within individual rats (r^2 0·72), BSS produced estimates that were 13 h longer than those based on CrEDTA. Neither marker detected a change in MTT with increasing RPS intake but, with both, the rate constant (k_1) for the 'largest mixing pool' declined significantly ($P < 0·001$) as dietary RPS concentration was changed from 0–240 g/kg.

Resistant starch: Short-chain fatty acids: Gut transit time: Thermophilic bacterial spores

Enhanced breath H_2 concentrations in response to ingestion of starchy food (Anderson *et al.* 1981) provided some of the earliest evidence that not all the starch in normal human foods is digested in the small bowel. Within a year, Englyst *et al.* (1982) identified a starch fraction in cornflakes which was resistant to α-amylase (*EC* 3.2.1.1) *in vitro* and this group went on to demonstrate that a variable fraction of starch in cereals (Englyst & Cummings, 1985), bananas (Englyst & Cummings, 1986) and potatoes (Englyst & Cummings, 1987) escaped small-bowel digestion in healthy ileostomists. The magnitude of this fraction, now termed resistant starch (RS), depends on several factors including botanical origin, processing and storage and is readily manipulated during food production (Würsch & Delcour, 1995). In addition to obvious implications for the assessment of the energy value of foods, the recognition that starch digestion is incomplete in the small bowel opened new areas for physiological research especially the consequences for large-bowel (LB) fermentation and function.

Potato tubers are rich in starch packed in characteristic spherical or semi-spherical granules with an amylose : amylopectin ratio of approximately 1 : 3. In their native state, these granules show substantial resistance to α-amylase *in vitro* (Englyst & Cummings, 1987) and consumption by rats leads to stimulation of LB fermentation (Demigné & Rémésy, 1982). These characteristics, and the ready availability of raw potato starch

(RPS), made it an attractive reference material for the EURESTA project, a European Union-funded Concerted Action to determine the physiological implications of the consumption of RS in man (Asp *et al.* 1996). The present study was designed to provide quantitative information on the escape of starch from the small bowel when RPS was included at several dose levels in the diet of rats. Further, investigations were undertaken of LB fermentation including the production of short-chain fatty acids (SCFA) in the caecum and disappearance of substrate between the terminal ileum and faeces. Since it was anticipated that there would be changes in gastrointestinal transit time (Calvert *et al.* 1989), attempts were made to estimate (1) whole-gut mean transit time (MTT) using two contrasting markers (CrEDTA) and *Bacillus stearothermophilus* spores (BSS)) given together in a single test meal and (2) LB transit time based on continuous dosing with Cr_2O_3 in the diet and using direct measurements on the caecum and colon.

Brief accounts of parts of this study have been published (Mathers & Carter, 1993; Mathers & Smith, 1993).

MATERIALS AND METHODS

Animals, diet and feeding regimen

Four experimental diets (PS1–PS4) were prepared which differed only in the proportions of maize starch and RPS (Table 1). Each diet contained adequate quantities of all nutrients known to be required by growing rats together with 2 g Cr_2O_3/kg diet as an indigestible marker. Twenty male specific-pathogen-free Wistar rats (initial weight 100 (SD 7·6) g) provided by the Comparative Biology Centre, University of Newcastle, were housed in individual metabolism cages. Five animals were allocated at random to each experimental diet and offered 15 g air-dry diet at approximately 09.00 hours daily. Uneaten food was removed each morning, dried and weighed. The animals were weighed at the start of the study and on Mondays, Wednesdays and Fridays thereafter.

Experimental protocol

After a 10 d adaptation period, uneaten food, faeces and urine were collected quantitatively in a 5 d balance period (Fig. 1). On day 15, the usual 09.00 hours feed was withheld until

Table 1. *Composition of experimental diets (g/kg)*

Diet code...	PS1	PS2	PS3	PS4
Maize starch*	240	160	80	0
Raw potato starch†	0	80	160	240
Sucrose	410	410	410	410
Casein	200	200	200	200
Cellulose‡	50	50	50	50
Maize oil	50	50	50	50
Vitamin premix§	15	15	15	15
Mineral premix‖	33	33	33	33
Cr_2O_3	2	2	2	2

* Happy Valley, Qualifry Ltd, London.
† Roquette, Lestrem, France: supplied by Dr M. Champ, INRA Nantes, France.
‡ Alphacel non-nutritive bulk (Cat. no. 900453) ICN Biochemicals, Ohio, USA.
§ AIN vitamin mixture 76A (Cat. no. 960098) ICN Biochemicals.
‖ AIN mineral mixture 76 (Cat. no. 905455) ICN Biochemicals.

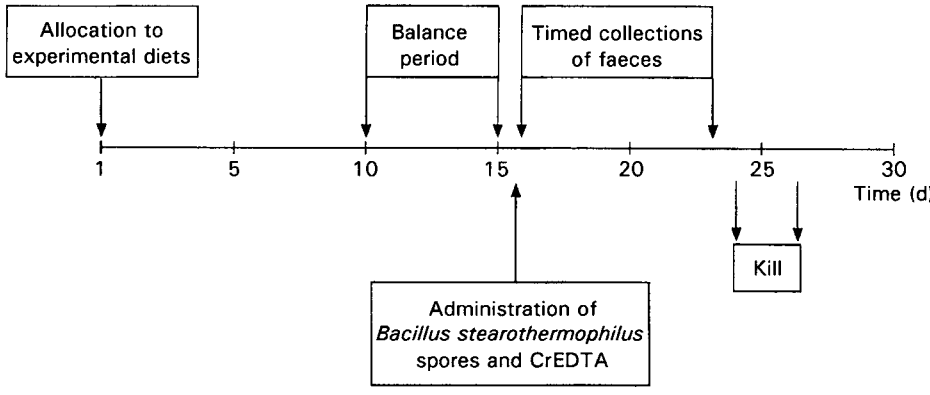

Fig. 1. Time-line for the study.

22.00 hours at which time 15 g diet mixed with the two experimental transit time markers (2 ml CrEDTA and 0·9 ml *B. stearothermophilus* spore suspension (10^8 spores/ml) was offered (details of preparation of markers follow). Most of this test meal was eaten within 1 h and any uneaten food was removed and stored at −20°.

Faeces collection for transit time determination. Faeces passed between 09.00 and 21.45 hours on day 15 were taken as the pre-marker samples and a further nineteen timed collections of faeces were made over the following 8 d. Four collections were made on each of days 16 and 17, two collections on days 18–22 with the final collection at 12.45 hours on day 23. Collected faeces were stored immediately at −20° before being freeze-dried and ground.

Preparation of transit time markers: (a) chromium EDTA. The method was based on that described by Binnerts *et al.* (1968). $CrCl_3.6H_2O$ (1·42 g) was dissolved in 20 ml distilled water whilst 2·0 g disodium EDTA was dissolved in 30 ml distilled water (required heating and stirring). The EDTA solution, together with two or three anti-bumping granules, was added to the Cr solution, covered with a watch glass and boiled gently for 1 h. 1 M-$CaCl_2$ solution (0·4 ml) was added and the pH adjusted to 6·2 by addition of NaOH solution (100 g NaOH/l). The resulting solution was made to 100 ml, mixed well and stored in plastic bottles.

(*b*) Bacillus stearothermophilus *spores.* Spores were purchased from Diagnostica Merck, Darmstadt, Germany (Cat. no. 11499). The suspension was centrifuged for 1 min at 13 000 rev./min in a microcentrifuge (Microcentaur, MSE, S. H. Scientific, Blyth, Northumberland), the supernatant fraction was removed by aspiration and the spores resuspended in distilled water to provide a concentration of 10^8 spores/ml.

Tissue, blood and digesta collections. Two animals from each diet were selected at random for slaughter on day 24 and the remaining three from each diet on day 26. At 2 h before sample collection, each animal was injected intraperitoneally with vincristine sulfate solution (providing 1 mg vincristine sulfate/kg body mass) for assessment of intestinal crypt cell proliferation (results not reported here). Approximately 110 min later, anaesthesia was induced by intraperitoneal injection of Hipnorm/Midazolam (0·1 ml/300 g body mass), and a mid-line laparotomy was performed. Samples of blood were collected from the portal vein (1 ml) and the heart (2–3 ml) into heparinized syringes and the liver removed, rinsed in ice-cold saline, blotted dry and weighed. The stomach was dissected out and weighed, its contents were removed and the tissue rinsed, blotted dry and weighed. The

small-intestine length was measured, the terminal sixth was cut off and the contents of the latter emptied into a 10 ml tube by flushing with 5 ml saline (9 g NaCl/l). Caecal mass was measured and the pH of its contents recorded using a microelectrode. Duplicate portions (0·6–0·8 g) of caecal digesta were weighed into microcentrifuge tubes and mixed with half the mass by volume of deproteinizing solution (metaphosphoric acid solution (200 g/l) containing 50 mM-3-methyl valeric acid) in preparation for SCFA determination (Mathers *et al.* 1990). The remaining caecal contents were transferred into pre-weighed vials and the tissue was rinsed, blotted dry and weighed. The colon was weighed and its length recorded. Colonic contents were removed into pre-weighed vials and the tissue rinsed, blotted dry and weighed.

Laboratory analyses

Diets, digesta and faeces. Digesta and faeces were freeze-dried and ground. Cr_2O_3 concentrations were determined as described by Mathers *et al.* (1990) and organic matter (OM) by heating at 500° for 16 h. Total starch was determined by enzymic hydrolysis to glucose (Englyst *et al.* 1992) and measurement of the latter by glucose dehydrogenase (*EC* 1.1.3.4) on a Cobas Mira clinical analyser (Roche, Welwyn Garden City, Herts.). For ileal samples, the procedure was scaled down by a factor of ten. Free glucose and α-linked glucose oligomers were included in the measurement of total starch. Caecal SCFA were determined by GLC (Mathers *et al.* 1990).

Transit time markers (*a*) Bacillus stearothermophilus *spores.* Freeze-dried and ground faecal samples were suspended in an appropriate volume of distilled water e.g. 80 mg in 2 ml, left overnight and then vortex mixed. Further serial dilutions of this suspension were made and 0·1 ml portions spread on nutrient agar plates before incubation at 65° for 16 h. The aim of the serial dilutions was to obtain counts of 30–300 colonies per plate and results were expressed as spores/g faecal DM. Preliminary studies showed that the BSS required temperatures of 55–65° for germination and that BSS added to faeces could be recovered quantitatively. To test for the presence of other thermophilic bacteria in the faeces of animals fed on these diets, samples of faeces from the balance period, i.e. before administration of the BSS spores (see Fig. 1), were processed as described earlier and incubated at 65° for 16 h.

(*b*) *Chromium EDTA.* Portions of the same faecal sample suspensions as used for spore counting were centrifuged for 15 min at 3000 rev./min (Mistral 3000i, S. H. Scientific) and Cr in the supernatant fraction was assayed by atomic absorption spectrometry at 357·9 nm using an acetylene flame (SP9, Pye Unicam Ltd, Cambridge, Cambs.). Results were expressed as concentrations of Cr per unit faecal DM. Cr_2O_3 provided in the diet is insoluble and is not measured by this procedure.

Calculations

Variables based on chromic oxide. Apparent digestibilities of each food consistent within individual gut compartments were calculated by the marker ratio method. Flow rates from the terminal ileum were based on analysis of digesta collected from the terminal sixth of the small intestine using the equation:

$$\text{flow rate of X (g/d)} = \frac{Cr_2O_3 \text{ intake (mg/d)}}{Cr_2O_3\text{:X ratio (mg } Cr_2O_3\text{/g X)}}.$$

Caecal and colonic transit times were calculated as described by Faichney (1975) and adapted for the rat by Goodlad & Mathers (1987):

$$\text{transit time (d)} = \frac{\text{amount of } Cr_2O_3 \text{ in organ (mg)}}{Cr_2O_3 \text{ intake (mg/d)}}.$$

Variables based on Bacillus stearothermophilus *spores and chromium EDTA.* Mean transit time (MTT) through the whole gut was estimated by a method which makes no assumptions about the kinetics of gut transit:

$$\text{MTT (h)} = \frac{\sum m_i t_i}{\sum m_i},$$

where m_i is the number of *B. stearothermophilus* spores (or quantity of CrEDTA) present in the faeces passed at time interval t_i (h) after dosing with the markers. In addition, estimates of a rate constant (k_1) were obtained as the slope of the line of best fit to the natural logarithm of faecal marker concentration with time for the descending portion of the curve (see Fig. 2) for each rat separately.

Statistical analyses

Datasets were examined by one-way ANOVA with partition of the 3 df for between-diets differences and associated sums of squares (ss) using orthogonal polynomials to describe the responses to inclusion of increments of RPS in the diet. The between-rats within-diets component (16 df) was used as the error term. For certain variables (caecal weights, ileal flows, disappearance in the LB and SCFA pool sizes) where the range of means was large and the variance appeared to increase with the mean, values were logarithmically transformed before analysis. Back-transformed means and pooled SEM in the transformed scales are presented for these variables in the tables. Curve fitting for marker concentration-with-time for each animal separately was carried out using Fig.P (Fig.P Software Corporation, Durham, NC, USA).

RESULTS

Food intake, growth and dimensions of organs

All animals ate almost all the food offered so that DM intakes were very similar for all four diets and no significant differences in body-weight gain were detected (Table 2). Including RPS in the diet had no effect on stomach weight or on small intestine length but produced marked effects in the LB. LB fermentation of starch is expected to yield about half the metabolizable energy (ME) yielded by starch digestion in the small bowel (Mathers, 1992). This is likely to result in approximately 9 % ME less for diet PS4 compared with PS1. The lack of effect of this reduction on growth may mask changes in body composition, which was not measured in the present study. With each increment of RPS intake, there was a corresponding increase in caecal mass so that when the diet contained 240 g RPS/kg, caecal mass was five times greater than for the diet containing a similar concentration of maize starch (PS1) (Table 2). This increase in mass was due, largely, to a greater mass of caecal digesta but this was accompanied by a fourfold increase in caecal tissue. Whilst there was no detectable change in colon length with diet, consumption of RPS was associated with significant ($P < 0{\cdot}01$) increases in whole colon and in colonic tissue masses (Table 2).

Table 2. *Food intake, weight gain and gastrointestinal tissue and contents masses of rats fed on semi-purified diets containing 0–240 g raw potato starch (RPS)/kg diet*†

(Mean values for five rats per diet with pooled standard error of the mean)

Diet code...	PS1	PS2	PS3	PS4	Pooled	Statistical significance of dietary effects	
RPS in diet (g/kg)...	0	80	160	240	SEM	Lin	Quad
DM intake (g/d)‡	14·0	14·1	14·1	14·0	0·22	NS	NS
Weight gain (g/d)‡	4·4	4·6	4·2	4·4	0·21	NS	NS
Stomach:							
Whole organ (g)	8·74	9·42	11·87	10·77	2·07	NS	NS
Tissue (g)	1·27	1·20	1·26	1·14	0·05	NS	NS
Digesta DM (g)	3·55	3·11	5·11	4·38	0·94	NS	NS
Small intestine length (m)	0·913	0·901	0·911	0·949	0·0233	NS	NS
Caecum:							
Whole organ (g)	1·87	4·15	6·06	9·45	0·03	***	*
Tissue (g)	0·37	0·66	0·96	1·39	0·03	***	NS
Digesta DM (g)	0·28	0·92	1·46	1·86	0·07	***	*
Colon:							
Length (m)	0·147	0·157	0·152	0·159	0·0053	NS	NS
Whole organ (g)	2·01	2·50	2·45	3·85	0·28	**	NS
Tissue (g)	1·06	1·15	1·17	1·41	0·06	**	NS
Digesta DM (g)	0·48	0·49	0·32	0·81	0·12	NS	NS

Lin, linear; Quad, quadratic, effects of dietary RPS concentration.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, NS $P > 0·05$.
† For details of diet, see Table 1.
‡ During 5 d balance period (see Fig. 1).

Starch supply to, and disappearance within, the large bowel

With diet PS1, which contained only maize starch (no RPS), the flow of starch from the terminal ileum was 0·04 g/d (Table 3) and corresponded with a digestibility within the small bowel of 0·99 for maize starch. Stepwise additions of RPS to the diet at the expense of maize starch produced strong linear increases in flows of DM and OM from the terminal ileum which could be accounted for by the increased ileal output of starch. With diet PS4 (all RPS), the digestibility of starch within the small bowel was calculated to be only 0·28 which indicates that 72 % of RPS escaped small-bowel digestion. However, measurement of starch in faeces indicated that most of the starch entering the LB disappeared within that organ. Whole-gut starch digestibility was 1·0 for the all-maize-starch diet (PS1) and declined linearly to 0·8 for the all-RPS diet (PS4). Amounts of DM, OM and starch disappearing within the LB increased linearly with increasing RPS intake (Table 3) and the quantities of starch and OM apparently fermented were similar.

Caecal fermentation

Total SCFA concentration increased strongly linearly from 64 to 105 mmol/kg caecal contents as RPS intake increased (Table 4). With the first two increments of RPS, there was a concomitant fall in caecal pH but pH increased with the highest RPS diet (PS4) despite the greater concentration of SCFA. There were distinct differences in the pattern of individual caecal SCFA (estimated as molar proportion) with changes in diet. Acetate was always the major SCFA present and increased linearly ($P < 0·01$) with higher intakes of RPS whilst the proportion of propionate remained unchanged. Including 80 g RPS/kg in the

Table 3. *Flows (g/d) of DM, organic matter (OM) and starch from the terminal ileum, disappearance in the large bowel (LB)† (g/d) and whole-gut apparent digestibility in rats fed on semi-purified diets containing 0–240 g raw potato starch (RPS)/kg diet‡*

(Mean values for five rats per diet with their pooled standard error of the mean)

Diet code...	PS1	PS2	PS3	PS4	Pooled	Statistical significance of dietary effects		
RPS in diet (g/kg)...	0	80	160	240	SEM	Lin	Quad	Dev
Ileal flow:								
DM	1·64	2·27	2·55	3·50	0·060	**	NS	NS
OM	0·90	1·65	1·91	2·90	0·054	***	NS	NS
Starch	0·04	0·74	1·25	2·20	0·088	***	***	*
Disappearance in the LB:								
DM	0·62	1·18	1·36	2·20	0·116	**	NS	NS
OM	0·18	0·64	0·66	1·65	0·127	***	NS	**
Starch	0·02	0·57	0·90	1·61	0·128	***	***	*
Whole-gut apparent digestibility:								
DM	0·93	0·93	0·92	0·91	0·005	**	NS	NS
OM	0·94	0·93	0·93	0·91	0·005	**	NS	NS
Starch	1·00	0·95	0·89	0·80	0·019	***	NS	NS

Lin, linear; Quad, quadratic; Dev, deviations from Lin and Quad effects of dietary RPS concentration.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, NS $P > 0·05$.
† Estimated as ileal flow rate − faecal output.
‡ For details of diets, see Table 1.

Table 4. *Caecal pH and short-chain fatty acids (SCFA) in rats fed on semi-purified diets containing 0–240 g raw potato starch (RPS)/kg diet†*

(Mean values for five rats per diet with pooled standard error of the mean)

Diet code...	PS1	PS2	PS3	PS4	Pooled	Statistical significance of dietary effects		
RPS in diet (g/kg)...	0	80	160	240	SEM	Lin	Quad	Dev
pH	6·7	6·4	6·2	6·5	0·08	NS	**	NS
Total SCFA (mmol/kg wet caecal contents)	64	72	95	105	10·6	**	NS	NS
Molar proportions of individual SCFA (mmol/mol)								
Acetate	611	587	658	686	21·3	**	NS	NS
Propionate	194	149	152	145	16·7	NS	NS	NS
Isobutyrate	18	12	3	2	1·3	***	NS	NS
Butyrate	123	208	169	146	20·6	NS	*	NS
Isovalerate	33	30	15	19	2·3	***	NS	*
Valerate	21	14	4	2	1·1	***	*	*
SCFA pool size (μmol/rat)								
Acetate	59	144	326	544	0·07	***	NS	NS
Propionate	18	37	74	108	0·07	***	NS	NS
Isobutyrate	2	3	1	1	0·14	NS	NS	NS
Butyrate	12	51	80	106	0·10	***	**	NS
Isovalerate	3	7	7	15	0·07	***	NS	*
Valerate	2	3	1	1	0·14	*	NS	NS
Total SCFA	96	246	496	795	0·07	***	NS	NS

Lin, linear; Quad, quadratic; Dev, deviations from Lin and Quad effects of dietary RPS concentration.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, NS $P > 0·05$.
† For details of diets, see Table 1.

diet resulted in a 70 % increase in caecal butyrate proportion but higher intakes of RPS led to progressively lower butyrate proportions. Molar proportions of the quantitatively minor SCFA (isobutyrate, isovalerate and valerate) all declined from diet PS1 to PS4.

Caecal SCFA pool sizes (calculated as the product of SCFA concentration in digesta and caecal digesta mass) increased approximately ninefold for both acetate and butyrate, sixfold for propionate and fivefold for isovalerate between the all-maize-starch and all-RPS diets (Table 3). Pool sizes of isobutyrate and valerate remained small and little changed by diet. The eightfold increase in total caecal SCFA pool size was largely a consequence of the large increase in caecal digesta contents (Table 2) as maize starch was exchanged for RPS.

Comparison of thermophilic bacterial spores and chromium EDTA as markers

With appropriate dilution, BSS could be identified and quantified in faecal suspensions following incubation on nutrient agar plates at 65 ° for 16 h. Colonies appeared as discrete raised white spots which were readily quantified when there were 30–300 colonies per plate. Thermophilic bacteria were absent from faeces collected during the balance period i.e. before dosing with BSS. The CV for counts between duplicate spread plates for the whole experiment was 9·6 %.

Following a single oral dose of both BSS and CrEDTA given in a test meal late one evening, the pattern of excretion of marker in faeces was similar. Faecal marker concentrations were low in the first sample collected the morning after dosing, rose rapidly to reach a maximum at about 17 h post-dosing and declined exponentially thereafter (Fig. 2). Although faeces collection continued until day 8 following marker administration, concentrations of both BSS and CrEDTA in faeces were very low from day 6 onwards making accurate determination for these late times difficult.

Estimates of whole-gut MTT were much greater for BSS (average 13 h longer) than for CrEDTA but no significant between-diet differences were detected by either marker (Table 5). Although there was a clear difference in estimates of MTT for the two markers, for individual rats the values obtained were strongly correlated:

$$\mathrm{MTT_{BSS}} = 9{\cdot}3\ (\mathrm{SD}\ 3{\cdot}54) + 1{\cdot}2\ (\mathrm{SD}\ 0{\cdot}18)\ \mathrm{MTT_{CrEDTA}}\ (r^2\ 0{\cdot}72).$$

A rate constant (k_1) was obtained as the gradient of $\log_e$ marker concentrations-with-time for the post-peak portion of the faecal excretion curves (Fig. 2 (a) and (b)). The goodness of fit, estimated as the correlation coefficient, for the fitted line was 0·94 or better for all rats for both markers except for one animals for BSS (r 0·81) and one for CrEDTA (r 0·87). For individual rats, estimates of k_1 for the two markers were strongly correlated:

$$k_1(\mathrm{BSS}) = 0{\cdot}02\ (\mathrm{SD}\ 0{\cdot}012) + 1{\cdot}26\ (\mathrm{SD}\ 0{\cdot}17)\ k_1(\mathrm{CrEDTA})\ (r^2\ 0{\cdot}75),$$

but were consistently higher for BSS than for CrEDTA (Table 5). As the quantity of RPS fed increased, there was a strong linear decrease in estimates of k_1 which was similar for both markers.

Large-bowel transit time estimates

Transit time (TT) of digesta in the caecum based on Cr_2O_3 measurements increased fourfold from 8·2 to 32·4 h as RPS replaced maize starch in the diet (Table 6). There were no significant diet effects on colonic TT with an overall mean of 9·2 h. The dominant effect

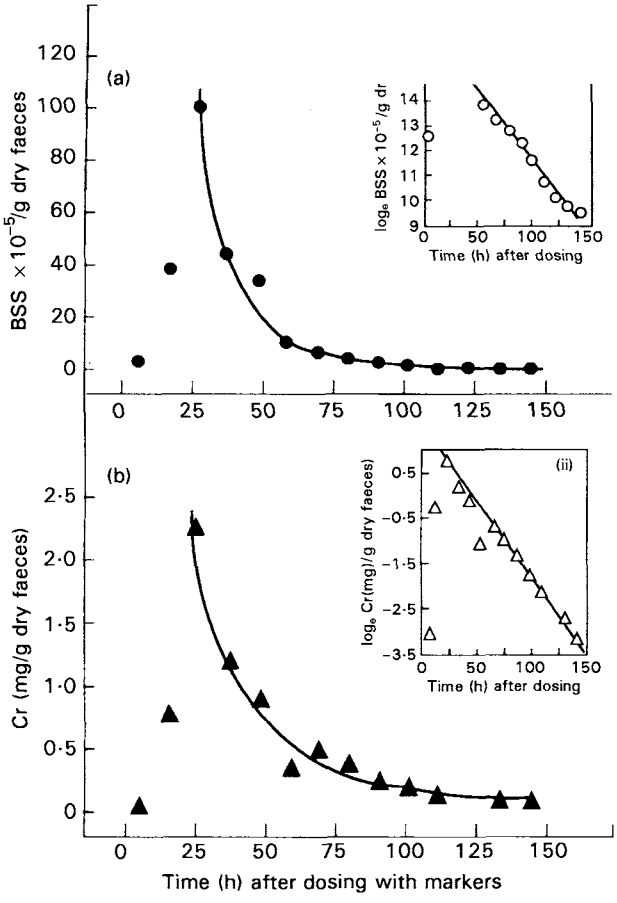

Fig. 2. Concentrations of (a) *Bacillus stearothermophilus* spores (BSS) (●—●) and (b) chromium-EDTA (▲—▲) in faeces following a single oral dose of the markers given in an test meal. This example is for a rat fed on diet PS2 (see Table 1). The inset panels illustrate the extraction of a rate constant (k_1) from the descending portions of the natural logarithm of the marker concentration-with-time curves for (i) BSS (○—○) and (ii) chromium-EDTA (△—△).

of the caecum in determining LB (caecum + colon) TT was apparent for all RPS-containing diets.

DISCUSSION

RS has been identified as 'the sum of starch and products of starch degradation not absorbed in the small intestines of healthy individuals' (Asp, 1992) and, by definition, identifies a carbohydrate fraction of food which flows to the LB. In its native form, potato starch exists as relatively large spherical or elipsoid granules which have the B-type powder diffraction pattern (Gallant *et al.* 1992). Scanning electron microscopy studies indicate that the granules are composed of more-or-less spherical blocklets which are 400–500 nm in diameter and at the surface of the granules these form a tough layer about 10 μm deep which confers resistance to enzymic hydrolysis (Gallant *et al.* 1992). In contrast, most cereal starches have smaller polyhedric granules with the A-type powder diffraction pattern which are less resistant to enzymic hydrolysis (Gallant *et al.* 1992). On this basis, we chose

Table 5. *Estimates of mean transit time (MTT, h) and of the rate constant* k_1 *obtained following a single oral dose of both* Bacillus stearothermophilus *spores (BSS) and chromium-EDTA markers in rats fed on semi-purified diets containing 0–240 g raw potato starch (RPS)/kg diet*†

(Mean values for five rats per diet with pooled standard error of the mean)

Diet code...	PS1	PS2	PS3	PS4	Pooled	Statistical significance of dietary effects
RPS in diet (g/kg)...	0	80	160	240	SEM	Lin
MTT:						
BSS	33·3	34·4	29·1	34·8	2·17	NS
CrEDTA	18·5	21·3	17·9	21·4	1·50	NS
Rate constant (k_1)‡:						
BSS	0·133	0·107	0·111	0·077	0·0072	***
CrEDTA	0·090	0·066	0·071	0·050	0·0046	***

Lin, linear effect of dietary RPS concentration. There were no significant quadratic or deviations from linear and quadratic effects of dietary RPS concentration.

*** $P < 0·001$, NS $P > 0·05$.

† For details of diet, see Table 1.

‡ For details of derivation, see p. 1019.

maize starch and RPS as contrasting starches to include in diets for rats with the objectives of (1) quantifying starch digestion in the small bowel and fermentation in the large bowel and (2) investigating the effects of increasing starch supply to the large bowel on aspects of fermentation and on intestinal transit time. All diets were of identical composition except for the proportions of maize starch (240–0 g/kg diet) and RPS (0–240 g/kg diet).

Starch digestion in the small bowel

Virtually all of the maize starch, but only 28 % of the RPS, was digested in the small bowel of these rats (Table 3). From an *in vitro* assay, 24 % of RPS was predicted to be digestible

Table 6. *Comparison of estimates of caecal and colonic transit time (TT) obtained from 'continuous' oral dosing with* Cr_2O_3 *in the diet with the reciprocal of the rate constant* (k_1) *obtained from single oral doses of both* Bacillus stearothermophilus *spores (BSS) and chromium-EDTA in rats fed on diets containing 0–240 g raw potato starch (RPS)/kg diet*†

(Mean values for five rats per diet with pooled standard error of the mean)

Diet code...	PS1	PS2	PS3	PS4	Pooled	Statistical significance of dietary effects
RPS in diet (g/kg)...	0	80	160	240	SEM	Lin
TT from Cr_2O_3:						
Caecum (h)	8·2	18·2	27·1	32·4	2·9	***
Colon (h)	11·6	10·1	10·5	10·5	2·0	NS
Large bowel (h)	19·8	28·3	42·8	42·8	4·0	*
$1/k_1$:						
BSS (h)	7·5	9·4	9·13	11·8	1·0	***
CrEDTA (h)	11·2	15·8	14·4	20·4	1·2	***

Lin, linear effects of dietary RPS concentration. There were no significant quadratic or deviations from linear and quadratic effect of dietary RPS concentration.

* $P < 0·05$, *** $P < 0·001$, NS $P > 0·05$.

† For details of diet, see Table 1.

in the human small bowel (Englyst *et al.* 1992). Later studies with human ileostomists showed that when RPS was incorporated into biscuits and given in a single test meal, all of the RS predicted by the same *in vitro* assay to be present in the biscuit was recovered in ileal effluent (Englyst *et al* 1996).

These observations suggest that, at least for this model food, estimates of small-bowel starch digestibility obtained by the *in vitro* assay (Englyst *et al.* 1992) and by the present *in vivo* rat assay are good predictors of starch digestibility in the human small bowel. It remains to be established whether the ileostomy model provides estimates of small bowel digestion which are quantitatively similar to those pertaining in the intact intestine. In a direct comparison between an intubation technique in healthy young (19–27 years) volunteers and the ileostomy model (mean age of volunteers 43 years), there was a significantly greater escape from the small intestine of starch from raw banana flour with the intubation procedure (Langkilde *et al.* 1995).

Fermentation in the large bowel

Replacing maize starch by RPS in the diet produced strong linear increases in flows of DM, OM and starch to the LB (Table 3) and resulted in marked hypertrophy of the caecum and, to a much lesser extent, of the colon (Table 2). This hypertrophy of the LB with RPS feeding has been reported before (Demigné & Rémésy, 1982; Calvert *et al.* 1989) and is probably an adaptive response to enable salvage of energy from fermentation of starch escaping small-bowel digestion (Wyatt *et al.* 1988; Goodlad & Mathers, 1990; Mathers & Dawson, 1991). If it is assumed that all of the starch reaching the LB with diets PS2–PS4 is RPS, then 77, 72 and 73 % respectively of this starch was fermented. This suggests that the fermentative capacity of the caecum was able to cope equally effectively with RPS over a wide range of intakes.

As anticipated, the greater fermentation in the caecum with increasing RPS intake was characterized by higher concentrations of SCFA (Table 4) but, although pH fell with the first two increments of RPS, with the highest increment caecal pH increased despite an accompanying rise in total SCFA concentration (Table 5). It is often assumed that faecal pH is determined largely by SCFA concentration (Wrong, 1995) but the present observation suggests that other factors, perhaps the presence of buffering agents or the formation of homo- or heterodimers (Fukushima, 1995), may also be important. Both *in vitro* (Englyst *et al.* 1987; Goodlad & Mathers, 1988) and *in vivo* (Mallet *et al.* 1988; Scheppach *et al.* 1988) studies have yielded evidence in support of the proposition that starch fermentation results in relatively high molar ratios of butyrate. Feeding a commercial 'instant' potato product to rats resulted in significantly greater OM flows to the LB than feeding freshly-cooked potatoes and was accompanied by higher molar proportions of butyrate in caecal contents (Mathers & Dawson, 1991). In the present study, raising RPS intake from zero to 80 g/kg diet produced a marked (70 %) increase in the molar proportion of butyrate (Table 4) but with further increases in RPS intake, butyrate proportion fell. This significant ($P < 0{\cdot}05$) curvilinear response may be important. It demonstrates that substrate supply is not necessarily the dominant factor determining SCFA pattern. This is in marked contrast with earlier experiments which found that increasing the intake of peas (*Pisum sativum*; Goodlad & Mathers, 1990), of wholemeal bread (Key & Mathers, 1993*a*) and of cooked haricot beans (*Phaseolus vulgaris*; Key & Mathers, 1995) produced linear increases in the molar proportion of butyrate. It is probable that NSP were the major component of the extra substrate supplied to the LB in these studies. Adding RPS to a starch-free diet for healthy human volunteers produced a 21 %

increase ($P < 0{\cdot}05$) in faecal butyrate molar proportion whereas wheat bran and RS from banana, wheat and maize were without significant effect (Cummings *et al.* 1996).

With all the RPS-containing diets (diets PS2–PS4) in the present study, availability of C will not have limited bacterial growth; indeed the increasing output of starch in the faeces shows that starch was in excess of the microflora's ability to utilize it despite a considerable increase in caecal size (Table 2) and a prolongation of transit time (Table 6). It has been argued that greater butyrate synthesis in conditions of substrate excess is a means of disposing of H so allowing regeneration of reduced dinucleotides and permitting glycolysis to proceed (Macfarlane, 1991; Mathers & Dawson, 1991). Other routes of H disposal may be operational with the high-RPS diets but these remain to be identified. Mathers & Dawson (1991) collated data on caecal TT and molar proportion of butyrate from four separate studies which used similar experimental methods and observed that caecal butyrate increased sharply when caecal TT decreased below about 0·75 d. The long caecal TT (up to 1·35 d with diet PS4) with the higher RPS intakes may have led to the selection of LB microbes favouring acetate production (Table 4). Despite the between-diets differences in SCFA molar proportions, the very large increase in caecal contents mass coupled with the increase in SCFA total concentration resulted in large linear rises in pool size for all three major SCFA. In contrast, molar proportions of the minor SCFA (isobutyrate, isovalerate and valerate) declined with increasing RPS intake possibly because of reduced amino acid fermentation (Macfarlane & Gibson, 1995) or utilization of these SCFA for bacterial protein synthesis (Russel & Hespell, 1981; Goodlad & Mathers, 1990).

Estimation of gastrointestinal transit time

Bacillus stearothermophilus *spores*. To our knowledge, this is the first report of the use of BSS in attempts to estimate MTT in the rat. Pochart *et al.* (1989) and Marteau *et al.* (1990) used BSS to estimate small-bowel transit in healthy human volunteers and the latter authors reported that (1) ileal output of the spores was similar to that of PEG 4000 included in the meal and (2) the proportion of spores recovered was high (99 (SE 10) %). In the present study, the overall pattern of excretion of BSS following a single oral dose was similar to that for Cr administered as the water-soluble CrEDTA (Fig. 2) with faecal concentrations falling to barely detectable levels after 6 d. However whole-gut MTT estimated by reference to BSS excretion was substantially longer than that for CrEDTA (Table 5). This was an unexpected finding since it has been reported that particulate and water-soluble markers pass through the non-ruminant gut at similar rates (Findlay *et al.* 1974; Luick & Penner, 1991). Despite this overall difference, within individual animals estimates of whole gut MTT for both markers were highly correlated.

Interpretation of rate constant (k_1). The descending portion of the faecal concentration-with-time curves was linear when plotted semi-logarithmically (Fig. 2). The slopes (rate constant k_1) of these portions of such curves have been assumed to represent the first order removal of marker from the 'largest mixing pool' within the gut (Grovum & Williams, 1973). In our rats, the stomach was the largest organ at the time of killing (4–6 h after feeding; Table 2) but, with once daily feeding, this would be expected to empty before the next meal unlike the caecum and colon (second and third largest gut components respectively) where diurnal variations in mass are much less marked (Mathers & Fotso Tagny, 1994). The reciprocal of k_1 provides an estimate of the TT of the marker through the compartment in question and this was compared with direct estimates of TT through the caecum and colon based on continuous dosing with Cr_2O_3 in the diet (Table 6).

There was no clear evidence that the pools characterized by the rate constant k_1 could be identified as either of the LB compartments. This may be because k_1 refers to another gut segment (or combination of gut segments) or because the marker Cr_2O_3 behaves differently with respect to movement in the gut from either BSS or CrEDTA. Further investigations will be needed before it becomes clear whether quantitative information on transit through individual gut components can be extracted from faecal concentration-with-time curves in the rat and so avoid the need for slaughter of the animals.

Effect of raw potato starch on gastrointestinal transit time. Estimates of whole-gut MTT obtained from a single oral dose of both BSS and CrEDTA were unaffected by diet (Table 5). However, estimates of caecal TT (based on continuous dosing with Cr_2O_3) and of TT through the putative largest mixing pool (based on both BSS and CrEDTA) all showed strong linear increases with increasing RPS intake (Table 6). Calvert *et al.* (1989) also reported that consumption of RPS by the rat increased intestinal TT estimated as the time required for 80% recovery of Cr_2O_3 following a single oral dose. The substantial increase in caecal TT is a function of the hypertrophy of that organ probably as a consequence of the enhanced substrate supply and the need to retain digesta and its microflora sufficiently long to allow salvage of energy from the starch escaping small-bowel digestion. This salvage became less complete the greater the intake of RPS which may be evidence for a compromise between the drive to maximize energy recovery from food by fermentation and the costs associated with caecal hypertrophy.

Conclusions

Over a wide range of intakes, approximately 70% of RPS escaped small-bowel digestion in the rat confirming the usefulness of RPS as a model material for studies of the physiological effects of RS. This extra substrate flowing to the LB stimulated fermentation in the caecum and resulted in substantial increases in SCFA production. Whilst 80 g RPS/kg diet provoked a significant increase in the molar proportion of butyrate in these SCFA, with higher RPS intakes the proportion of butyrate declined. These observations suggest that it will be important to consider dose–response effects carefully if the intention is to use RPS to induce an increased contribution of butyrate to SCFA production. At all levels of RPS intake, approximately three-quarters of the starch reaching the LB was fermented in that organ aided perhaps by the marked increases in both caecal size and TT. When peas (Goodlad & Mathers, 1990), wholemeal bread (Key & Mathers, 1993*a*) and haricot beans (Key & Mathers, 1993*b*, 1995) were provided over a wide range of intakes to supply mainly NSP as the source of additional substrate for the LB, there was also no evidence of any diminution of the capacity to ferment NSP at the higher intakes. In contrast with RPS feeding, caecal TT tended to fall with increasing NSP intake (see also Key *et al.* 1996) which may imply that RPS is more slowly fermented that is NSP from these sources.

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