Large bowel fermentation in rats eating processed potatoes

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Four diets were prepared in which cooked potatoes provided approximately 0·8 of the dry matter (DM) and all the polysaccharides. The potatoes were either boiled conventionally (unprocessed) or prepared by rehydrating a commercial 'instant' potato product with boiling water. The diets were fed to rats (six per diet) immediately after preparation or after storage at 4° for 48 h and observations made on site and extent of digestion and large bowel (LB) fermentation. All diets were equally well digested (overall means 0·95 and 0·96 for DM and organic matter (OM) respectively) with a significant proportion of this digestion occurring in the LB. More OM flowed to the LB with the 'instant' (0·18 of intake) than with the unprocessed potato diets (0·13 of intake) and was associated with markedly different fermentation patterns. When compared with rats fed on the unprocessed potato diets, those given 'instant' potatoes had smaller caecums with much shorter transit times, lower caecal pH, higher total volatile fatty acid (VFA) concentrations and a greater molar proportion of butyrate in these VFA. Storage of the diets for 48 h at 4° had relatively smaller effects on these variables. Possible mechanisms for the observed effects and implications for tissue metabolism and health are discussed. In addition, a simple and apparently novel method for quantifying coprophagy is proposed.

Large bowel fermentation: Potato processing: Starch digestion

The potato is an international staple crop which has now spread to at least three-quarters of the world's countries from its origins in South America (Woolfe, 1987). The tuber is rich in carbohydrates, the principal of which is starch present as granules consisting of amylopectin and amylose in the ratio of approximately 3:1. In their native state, these starch granules show considerable resistance to pancreatic α-amylase (*EC* 3.2.1.1; Englyst & Cummings, 1987*a*), escape small intestinal digestion and stimulate large bowel (LB) fermentation (Demigné & Rémésy, 1982). For this reason, potatoes are normally processed by some form of heating before consumption by man. The cooked potatoes may be eaten hot or sometime later when cold.

The present experiment was designed to investigate, in the rat, the effects on site and extent of digestion and on LB fermentation of feeding diets in which potatoes provided most (0·8) of the dry matter (DM) and all the polysaccharides. Studies using breath hydrogen as an index of LB fermentation (Anderson *et al.* 1981; Flourié *et al.* 1988) and direct measurements of flow through the terminal ileum using ileostomate volunteers (Chapman *et al.* 1985; Englyst & Cummings, 1985, 1986, 1987*a*) and intact healthy volunteers in whom a multi-lumen tube was positioned close to the ileo-caecal junction (Stephen *et al.* 1983; Flourié *et al.* 1988) have all indicated that a significant but variable proportion of the starch in various foods escapes small intestinal digestion and flows to the LB where it is presumed to be fermented. As yet, there is relatively little information on the effects of starch in the LB. Some of this starch escaping small intestinal digestion may be physically inaccessible to pancreatic α-amylase whilst some will have been rendered resistant due to retrogradation (resistant starch; RS) which occurs when a cooked, starch-

Table 1. *Composition of mineral and vitamin premix*

Mineral	Per kg premix	Vitamin	Per kg premix
$CaHPO_4$	340 g	Rovimix AD_3 500/100*	200 mg
NaCl	26 g	Rovimix E_{50} adsorbate*	1·2 g
$FeSO_4.7H_2O$	2·4 g	Menadione	10 mg
$ZnSO_4.7H_2O$	1·1 g	Choline chloride	27 g
$MnSO_4.4H_2O$	4·0 g	Calcium pantothenate	100 g
KIO_3	5·0 mg	Folic acid	15 mg
		Riboflavin	40 mg
Cr_2O_3†	40 g	Thiamin	20 mg
		Vitamin B_{12}	1 mg
Sucrose	557·9 g		

* Roche.
† Gastrointestinal flow marker.

rich product is allowed to cool (Englyst & Cummings, 1987*b*). In the present study we compared conventionally boiled potatoes with a commercial 'instant' product and fed each type of potato to rats when freshly prepared or after storage at 4° for 48 h. We anticipated that such storage would increase the amount of RS present which might influence substrate flow to the LB with subsequent effects on fermentation in that organ.

MATERIALS AND METHODS

Diets and feeding

Four diets were used in which potatoes provided approximately 0·8 of DM intake. For diets 1 and 2, unprocessed potatoes (var. Home Guard; A. S. McLaren & Son, Keillow, Methuen, Perthshire) were peeled, boiled for 20 min in tap water (without added salt), drained thoroughly, mashed immediately and 1 kg freshly mashed potato was mixed with 50 g supplement containing (g/kg) casein 485, maize oil 250, mineral and vitamin premix (Table 1) 250 and L-methionine 15. This mixture was fed either immediately (diet 1) or after storage at 4° for 48 h (diet 2). Diets 3 and 4 were prepared by mixing instant mashed potato (Yeoman brand; Dornay Foods, PO Box 15, Kings Lynn, Norfolk PE30 4JE) and supplement in the ratio 4:1 (w/w). When required, 225 g of this mixture was stirred into 900 ml boiling tap water and fed either immediately (diet 3) or after storage at 4° for 48 h (diet 4). Thus, all four diets contained approximately 0·2 DM as fed and each animal was offered 60 g moist diet daily at 10.00 hours. Food residues were removed at 14.00 hours. Animals had *ad lib.* access to water.

Animals and housing

Twenty-four male Wistar rats, initial weight 150 (SD 5·4) g, were divided into four dietary groups each of six animals and housed in individual metabolism cages (Thompson, 1970).

Measurements

After 10 d adaptation, food intake and faecal and urinary output were measured for 7 d. Each day, samples of the diets offered were dried at 100° as were all food residues to obtain a measure of DM intake. Faeces were collected daily, composited for each animal and stored at −20° before being freeze-dried and ground for analysis. Urine was collected into flasks containing 2 ml 6 M-hydrochloric acid.

Between 14.00 and 17.00 hours, rats were anaesthetized with diethyl ether, a mid-line

laparotomy was performed to expose the abdominal organs and the stomach, small intestine, caecum, colon and liver were dissected out. The terminal one-sixth by length of the small intestine was excised and the contents collected by flushing through with 5 ml saline (0·9 g sodium chloride/l). The caecum was weighed and the pH of its contents measured using a micro-electrode. Duplicate weighed samples (approximately 1 g) of caecal contents were mixed 2:1 (w/v) with deproteinizing solution (metaphosphoric acid solution (200 g/l) containing 50 mM-3-methyl valeric acid) in preparation for volatile fatty acid (VFA) determinations. Further samples of caecal contents were transferred into preweighed tubes for determination of DM and chromic oxide contents. The caecal tissue was washed, blotted dry and weighed. The stomach and colon were treated as for the caecum except that pH and VFA were not measured. The liver was rinsed in saline, blotted dry and weighed.

Analysis

VFA in caecal contents were measured by gas–liquid chromatography using a 2 mm i.d. column packed with 10% SP-1200/1% phosphoric acid on 80/100 Chromosorb (Supelco Inc., Bellefonte, Pennsylvania 16823, USA) in a Series 204 gas–liquid chromatograph (Pye Unicam, Cambridge). Samples of diet, faeces and of stomach, small intestinal, caecal and colonic contents were freeze-dried and ground before analysis. Subsequently for determination of DM, organic matter (OM) and Cr_2O_3 contents, portions (60–100 mg) were weighed into predried and preweighed graduated Pyrex tubes, dried at 100° for at least 5 h, re-weighed, ashed at 450° for 16 h and re-weighed. Two or three anti-bumping granules were added and the residue was digested using 1·2 ml acid mixture (300 ml $MnSO_4.4H_2O$ solution (100 g/l) diluted to 1 litre with orthophosphoric acid) and 1·6 ml potassium bromate solution (45 g $KBrO_3$/l) and made up to 10 ml with distilled water. To portions of the digest, 1 ml calcium chloride solution (5·47 g $CaCl_2.6H_2O$/l) and 0·1 ml sodium silicate solution (7·55 g $Na_2SiO_3.5H_2O$/l) were added and, after appropriate dilution, the chromium concentration was measured by atomic absorption spectrophotometry (SP9; Pye Unicam, Cambridge, UK).

For total starch determination, 500 mg portions of diet were treated with 1 ml dimethyl sulphoxide for 5 min at room temperature, 10 ml 0·1 M-sodium acetate buffer (pH 5·2) was added, the tubes were capped and heated in a boiling water-bath for 1 h. The tube contents were allowed to cool to approximately 50°, 0·2 ml amyloglucosidase (*EC* 3.2.1.3; Sigma Chemical Co. Ltd) was added and the samples incubated at 55° overnight. The resulting glucose was measured by a glucose dehydrogenase (*EC* 1.1.3.4) method (MA Kit 100; Roche) using a Cobas Mira clinical analyser (Roche). Readily digested starch was measured by incubating 500 mg diet suspended in 10 ml saline with 100 μl pancreatin solution (100 mg pancreatin (4 × NF Grade; Sigma)/ml saline) with constant stirring at 37° for 2 h. Undigested starch was precipitated with aqueous ethanol (850 ml/l), washed with a further 40 ml aqueous ethanol (850 ml/l), dried with acetone and assayed as described previously for total starch.

Calculations

Apparent digestibility and flow-rate from the terminal ileum were calculated by marker-ratio methods. Caecal and colonic transit times (TT) were calculated as:

$$\frac{\text{mass of } Cr_2O_3 \text{ in organ (mg)}}{\text{daily } Cr_2O_3 \text{ intake (mg/d)}},$$

as described by Faichney (1975) and Goodlad & Mathers (1987).

An estimate of the extent of coprophagy was obtained from knowledge of the concentration of Cr_2O_3 in the DM in the diet (D), in faeces (F) and in stomach contents

(S). Assuming that the sources of stomach DM were food and faeces, the proportion of stomach contents obtained directly from ingested food was:

$$\frac{S-F}{D-F}.$$

DM and OM disappearances in the LB were calculated as ileal flow-rate minus faecal output.

Estimates of VFA absorption were obtained from knowledge of caecal VFA proportions and of OM disappearance in the LB on the assumption that anhydrous hexose was the substrate fermented, i.e. $C_6 = A/2 + P/2 + B$, where C_6 is mol hexose fermented and A, P and B are mol acetate, propionate and butyrate absorbed (Demeyer & Van Nevel, 1975).

Statistical analysis

Data were examined by one-way analysis of variance in which the 3 df and accompanying sums of squares associated with between-diets differences were partitioned into single df orthogonal contrasts as follows: contrast 1, unprocessed potato *v.* 'instant' potato; contrast 2, freshly-prepared diets *v.* stored diets; contrast 3, (unprocessed potato *v.* 'instant' potato) × (freshly-prepared diets *v.* stored diets), i.e. the interaction between potato type and storage. In each case, the contrast mean square was compared with the between-animals within-diets mean square (which had 20 df) using an *F* test.

The relationship between the molar proportion of butyrate in VFA in caecal contents and caecal transit time in Fig. 1 is indicated by an hyperbola fitted using the maximum likelihood program (Ross, 1980). This curve was chosen for descriptive reasons since it accounted for a considerably higher proportion (0·60) of the variation in caecal butyrate than did the alternatives tested, i.e. exponential (0·30), power (0·40), logarithmic (0·43) and quadratic (0·46) curves.

RESULTS

Food intake, growth and DM digestibility

Rats on all diets lost weight during the first 5 d of the experiment whilst they became accustomed to eating these low-energy-density diets during the 4 h daily that food was available to them. One animal was replaced because of persistently low intakes. Thereafter, the animals regained the lost weight and during the final 7–9 d showed modest rates of weight gain which were not significantly different between diets (Table 2), but the relatively large between-animal within-diets variation should be noted. Significantly ($P < 0·001$) greater amounts of the 'instant'-potato diets compared with unprocessed-potato diets were eaten, as were diets stored for 48 h at 4° before feeding compared with freshly-prepared diets; the latter effect was due mainly to the relatively low intake of the freshly-prepared, unprocessed-potato diet (diet 1). For all diets, faeces were scanty with wet, poorly-formed pellets which made total collection unreliable. Consequently, all estimates of digestibility were based on the marker-ratio method. DM apparent digestibility was very similar for all diets with an overall mean of 0·95 and a relatively low coefficient of variation of 0·07. Rats eating the 'instant'-potato diets excreted slightly, but not significantly ($P > 0·05$), more urine than those eating the unprocessed-potato diets, perhaps because of the higher intakes and greater salt content of the former.

Tissue weights

At slaughter, the rat's stomachs appeared very full and contained approximately 25 g contents, equivalent to 0·13 of live weight. There were no significant effects of diet on masses of stomach or colon, but caecal mass was 1·5 times greater with unprocessed-potato diets when compared with 'instant'-potato diets (Table 3). This caecal hypertrophy was

Table 2. *Weight changes, dry matter (DM) intakes and apparent digestibilities and urinary output of rats offered cooked potato-based diets in which the potatoes were unprocessed or 'instant' and fed fresh or after storage at 4° for 48 h*

(Values are means for six animals)

	Unprocessed		'Instant'			Statistical significance of dietary effects‡		
Diet no.†...	Fresh 1	Stored 2	Fresh 3	Stored 4	SE of mean	Unprocessed *v.* 'instant'	Fresh *v.* stored	Interaction
Initial wt (g)	151	150	151	151	3·0	NS	NS	NS
Wt gain (g/d)§	1·4	1·8	1·9	2·0	0·27	NS	NS	NS
DM intake (g/d)‖	7·69	9·09	9·71	10·18	0·379	***	*	NS
DM apparent digestibility‖	0·948	0·949	0·954	0·946	0·026	NS	NS	NS
Urine output (g/7 d)‖	175	179	215	225	16·0	NS	NS	NS

* $P < 0·05$, *** $P < 0·001$.
NS, not significant.
† For details of composition, see p. 314 and Table 1.
‡ For details of orthogonal contrasts, see p. 316.
§ Prefeeding weights measured over final 7–9 d of experiment.
‖ Measured over 7 d of balance period.

Table 3. *Gastrointestinal organ, tissue and contents weights of rats offered cooked potato-based diets in which the potatoes were unprocessed or 'instant' and fed fresh or after storage at 4° for 48 h*

(Values are means for six animals, except where indicated)

	Unprocessed		'Instant'			Statistical significance of dietary effects‡		
Diet no.†...	Fresh 1	Stored 2	Fresh 3	Stored 4	SE of mean	Unprocessed *v.* 'instant'	Fresh *v.* stored	Interaction
Stomach								
Total organ (g)	25·9	26·9	25·8	26·8	1·93	NS	NS	NS
Stomach tissue (g)	1·39	1·43	1·45	1·41	0·092	NS	NS	NS
Contents (g): Wet	24·5	25·3	24·3	25·4	1·90	NS	NS	NS
Dry	4·39	4·69	4·46	4·74	0·321	NS	NS	NS
Caecum								
Total organ (g)	6·20	6·24	4·13	4·19	0·594	***	NS	NS
Caecal tissue (g)	0·95	0·91	0·78	0·86	0·058	***	NS	**
Contents (g): Wet	5·25	5·34	3·35	3·34	0·557	***	NS	NS
Dry	0·69	0·70	0·48	0·40	0·084	**	NS	NS
Colon								
Total organ (g)	1·52	1·73	1·47	1·91	0·206	NS	NS	NS
Colonic tissue (g)	0·74	0·76	0·80	0·89	0·051	NS	NS	NS
Contents (g): Wet	0·78	0·97	0·66	1·02	0·177	NS	NS	NS
Dry	0·12	0·16§	0·12	0·21	0·069	NS	NS	NS

NS, not significant.
** $P < 0·01$, *** $P < 0·001$.
† For details of composition, see p. 314 and Table 1.
‡ For details of orthogonal contrasts, see p. 316.
§ Five rats only.

observed for both caecal tissue and contents. For caecal tissue, there was a significant ($P < 0.01$) interaction between processing method and storage, with an increase in tissue mass in rats eating the 48 h stored 'instant'-potato diet but a decrease in those animals eating the stored unprocessed-potato diet compared with rats eating the equivalent freshly-prepared diets. Liver weights were unaffected by diet with means of 7·3, 7·1, 7·7 and 7·8 (SE 0·35) g for diets 1, 2, 3, and 4 respectively.

Caecal fermentation

Storage of the diets at 4° for 48 h had no effect on caecal fermentation pattern but feeding 'instant' potatoes significantly reduced caecal pH and increased caecal total VFA concentration when compared with unprocessed potatoes (Table 4). Caecal VFA pool sizes tended to be lower in animals eating the 'instant'-potato diets, because of the much smaller caecal contents masses (Table 3), but these differences were not statistically significant. There was a significantly higher proportion of butyric acid with lower proportions of propionic and isovaleric acids in caecal VFA from rats fed on 'instant'-potato diets, whilst diet had no significant ($P > 0.05$) effects of molar proportions of acetic, isobutyric and valeric acids.

Coprophagy

Knowledge of the Cr_2O_3 concentrations in food, stomach contents and faeces allowed calculation of the proportions of stomach contents DM derived from food and faeces (assuming that these were the only significant sources of stomach contents DM). At the time of killing, it was calculated that directly ingested food contributed 0·99, 0·99, 0·99 and 1·00 (SE 0·005) of stomach contents DM for diets 1, 2, 3 and 4 respectively.

Extent and sites of digestion of organic matter

The higher DM intakes of animals eating the 'instant'-potato diets (Table 2) were reflected in the higher OM intakes reported in Table 5, with a significantly lower intake of the fresh compared with stored diets largely as a result of the relatively low intake for diet 1. Diet had no significant effect on OM apparent digestibility with an overall mean value of 0·96 associated with a relatively low coefficient of variation of 0·06. OM flow from the terminal ileum was considerably greater for the 'instant'-potato diets compared with unprocessed, and for the stored compared with freshly-prepared diets. The amount of OM apparently fermented in the LB, calculated as the difference between OM flow from the ileum and OM output in faeces, was very highly significantly ($P < 0.001$) greater for animals eating the 'instant'-potato diets than for the others.

Since some of these effects in gastrointestinal OM flow may have been due to the differences between diets in OM intake, the values were also expressed per kg OM intake. On this basis, OM flow from the terminal ileum into the LB with the 'instant'-potato diets was, on average, 1·4 times that observed with the unprocessed-potato diet and, since OM output in faeces was unaffected by diet, there was a similar increase in the amount of OM apparently fermented in the LB.

LB transit times

Transit times (TT) of the marker Cr_2O_3 in the caecum were approximately twice as long in rats eating unprocessed-potato diets compared with the 'instant'-potato diets, and storage of the diet at 4° for 48 h before feeding reduced caecal TT by approximately one-third for each potato type (Table 6). Colonic TT was much shorter than caecal TT but, again, was significantly ($P < 0.001$) longer for the unprocessed-potato-fed animals than for those eating 'instant' potatoes.

Table 4. *pH, total volatile fatty acids (VFA) and molar proportions of individual VFA in caecal contents of rats offered cooked potato-based diets in which the potatoes were unprocessed or 'instant' and fed fresh or after storage at 4° for 48 h*

(Values are means for six animals)

	Unprocessed		'Instant'			Statistical significance of dietary effects‡		
Diet no.†...	Fresh 1	Stored 2	Fresh 3	Stored 4	SE of mean	Unprocessed *v.* 'instant'	Fresh *v.* stored	Interaction
Caecal pH	6·4	6·2	6·2	6·1	0·10	*	NS	NS
Total VFA (mmol/kg caecal contents)	116	102	180	145	12·7	***	NS	NS
Caecal VFA pool (mmol/rat)	0·64	0·52	0·57	0·48	0·076	NS	NS	NS
Molar proportions of individual VFA (mmol/mol):								
Acetic acid	666	679	667	687	14·7	NS	NS	NS
Propionic acid	207	189	138	151	17·6	**	NS	NS
Isobutyric acid	2	3	2	3	0·6	NS	NS	NS
Butyric acid	100	106	178	146	23·0	*	NS	NS
Isovaleric acid	17	17	8	8	2·0	***	NS	NS
Valeric acid	8	7	7	6	1·0	NS	NS	NS

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of composition, see p. 314 and Table 1.
‡ For details of orthogonal contrasts, see p. 316.

Table 5. *Partition of digestion of organic matter (OM) between the small and large bowels of rats offered cooked potato-based diets in which the potatoes were unprocessed or 'instant' and fed fresh or after storage at 4° for 48 h*

(Values are means for six rats)

	Unprocessed		'Instant'			Statistical significance of dietary effects‡		
Diet no.†...	Fresh 1	Stored 2	Fresh 3	Stored 4	SE of mean	Unprocessed *v.* 'instant'	Fresh *v.* stored	Interaction
OM intake (g/d)	7·46	8·80	9·21	9·51	0·364	**	*	NS
OM flow from ileum (g/d)	0·94	1·10	1·46	1·87	0·127	***	*	NS
OM output in faeces (g/d)	0·30	0·36	0·34	0·43	0·103	NS	NS	NS
OM apparently fermented (g/d)§	0·65	0·74	1·12	1·43	0·120	***	NS	NS
OM apparent digestibility	0·960	0·959	0·963	0·955	0·023	NS	NS	NS
OM flow from ileum (g/kg OMI)	124	126	158	196	13·3	***	NS	NS
OM output in faeces (g/kg OMI)	40	41	37	45	2·3	NS	NS	NS
OM apparently fermented (g/kg OMI)	84	85	121	150	12·9	***	NS	NS

NS, not significant; OMI, organic matter intake.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of composition, see p. 314.
‡ For details of orthogonal contrasts, see p. 316.
§ Calculated as OM flow from ileum − OM output in faeces.

Estimated absorption of VFA from the LB

VFA absorbed from the LB were calculated from knowledge of the OM apparently fermented in this organ, i.e. OM disappearance between the terminal ileum and faeces (Table 5) and the molar proportions of the major VFA (acetate, propionate and butyrate) observed in the caecum (Table 4) using conventional anaerobic stoichiometry i.e. that one molecule of hexose can result in two molecules of acetate, two molecules of propionate or one molecule of butyrate (Demeyer & Van Nevel, 1975), and assuming that these VFA were the only significant organic end-products of fermentation. Given the differences in OM intake (Table 5), values for VFA absorption are reported both in absolute amounts (mmol/d) and also per kg OM intake (Table 7). Both methods of expression resulted in similar conclusions about the effects of diet. On a molar basis, acetate was the major acid apparently absorbed with significantly greater amounts being absorbed with the 'instant'-potato diets compared with unprocessed-potato diets. There also tended to be more propionate absorbed with the 'instant'-potato diets but between-diets differences were not statistically significant ($P > 0{\cdot}05$). When expressed as mmol per kg OM intake, there was only half as much butyrate as propionate absorbed with the unprocessed-potato diets but similar molar amounts of propionate and butyrate with the 'instant'-potato diets. Total VFA absorption was significantly ($P < 0{\cdot}01$) greater with the 'instant'-potato diets.

DISCUSSION

Experimental objectives and protocol considerations

The present experiment was designed to investigate effects on the site and extent of OM digestion and LB fermentation of feeding diets in which 0·8 of the DM was provided by cooked potatoes (the only dietary source of polysaccharides), and which were prepared either by the conventional boiling method or by rehydrating commercially-prepared 'instant' potatoes. Since the variety of potato used in manufacture of the 'instant' potato is not known, it should be borne in mind that differences attributed to potato type (unprocessed *v.* 'instant') may in part be due to inherent differences in the potato varieties. A further objective was to investigate the effects of refrigeration (4°) of the diets for 48 h before feeding, since the practice of keeping cooked potato cold for some time before eating is widespread in domestic, institutional and commercial catering. Such storage is likely to result in the formation of a starch fraction which is resistant to α-amylase (RS; Englyst & Cummings, 1987*a*, *b*).

The experimental objectives resulted in practical difficulties not usually encountered in nutritional experiments with rats. First, potatoes had to be cooked and diets prepared every day, which could have resulted in some between-day variation in composition. This was minimized by adhering strictly to the diet preparation protocol and portions of each day's diet were pooled and analysed to provide a best estimate of diet composition. Second, the diets were high in water content (0·8) and of low energy density so that large volumes had to be consumed to meet the rats' nutrient requirements. Since it was important to minimize the formation of RS in the freshly-prepared diets (to provide a contrast with the stored diets), it was necessary to train the rats to consume as much as possible rapidly after the food was offered, and a 4 h period (10.00–14.00 hours daily) was allowed for this purpose.

Several interesting differences (discussed later) were observed between the 'instant'- and unprocessed-potato diets, but fewer and less marked differences between the freshly-prepared and stored diets. This may have arisen because the 4 h during which the rats had access to the freshly-prepared diets may have been long enough for retrogradation of some starch to form RS, as would also have occurred in the cold-stored diets, so reducing potential differences between the diets. After the present study had been completed, further portions of the 'instant'-potato diet (using the same batch of rat materials) and of the fresh-

Table 6. *Transit times (d) in the caecum and colon of rats offered cooked potato-based diets in which the potatoes were unprocessed or 'instant' and fed fresh or after storage at 4° for 48 h*

(Values are means for six animals).

	Unprocessed		'Instant'			Statistical significance of dietary effects‡		
Diet no.†...	Fresh 1	Stored 2	Fresh 3	Stored 4	SE of mean	Unprocessed *v.* 'instant'	Fresh *v.* stored	Interaction
Caecum	1·51	1·08	0·79	0·47	0·165	***	*	NS
Colon	0·29	0·26	0·18	0·21	0·053	***	NS	NS

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of composition, see p. 314 and Table 1.
‡ For details of orthogonal contrasts, see p. 316.

Table 7. *Estimates of volatile fatty acid (VFA) absorption† from the large bowel of rats offered cooked potato-based diets in which the potatoes were unprocessed or 'instant' and fed fresh or after storage at 4° for 48 h*

(Values are means for six animals)

	Unprocessed		'Instant'			Statistical significance of dietary effects§		
Diet no.‡...	Fresh 1	Stored 2	Fresh 3	Stored 4	SE of mean	Unprocessed *v.* 'instant'	Fresh *v.* stored	Interaction
Acetate (mmol/d)	4·9	5·7	8·1	10·8	0·98	***	NS	NS
Propionate (mmol/d)	1·5	1·6	1·7	2·5	0·38	NS	NS	NS
Butyrate (mmol/d)	0·8	0·9	2·0	2·2	0·37	**	NS	NS
Total VFA (mmol/d)	7·2	8·2	11·8	15·5	1·37	***	NS	NS
Acetate (mmol/kg OMI)	640	660	880	1130	107	**	NS	NS
Propionate (mmol/kg OMI)	200	180	190	260	42	NS	NS	NS
Butyrate (mmol/kg OMI)	100	100	210	230	30	***	NS	NS
Total VFA (mmol/kg OMI)	940	940	1290	1620	140	**	NS	NS

NS, not significant; OMI, organic matter intake.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of calculation, see pp. 315–316.
‡ For details of composition, see p. 314 and Table 1.
§ For details of orthogonal contrasts, see p. 316.

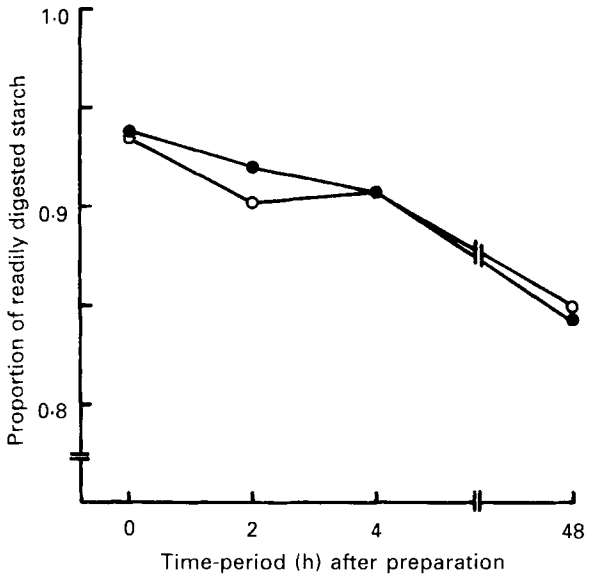

Fig. 1. Readily digested starch as a proportion of total starch in samples of unprocessed-potato (var. Pentland Squire) (●—●) and 'instant'-potato (○—○) diets at 0, 2, 4 and 48 h after preparation. Points are means of two determinations. For details of diets and procedures, see pp. 314–316.

potato diet (using the var. Pentland Squire) were prepared and assayed for total and readily digested starch immediately, and at 2, 4 and 48 h after preparation. The 48 h sample was stored at 4° as in the main experiment. As expected, the proportion of starch which was readily digested by pancreatin fell with time after preparation with very similar values for both the unprocessed and 'instant'-potato diets (Fig. 1).

Sites and extent of digestion

Overall mouth-to-faeces digestibility of both DM and OM were high, as expected, and did not differ between diets. With all diets, a considerable proportion (0·12–0·20) of the ingested OM flowed from the terminal ileum but most of this (0·67–0·77) disappeared within the LB, presumably as a result of fermentation. The flow of fermentable material into the LB of normal healthy human subjects given microwave-cooked potato has been assessed by the H_2 excretion method and found to correspond to approximately 0·08–0·10 of ingested carbohydrate (Levitt *et al.* 1987), in good agreement with our observations given in Table 5. The composition of this OM escaping digestion in the small bowel has not been examined in detail, but preliminary investigations suggest that starch may account for only 0·15–0·21 of the OM flowing into the LB (J. C. Mathers & L. D. Dawson, unpublished results) which would correspond to approximately 0·04–0·06 of the ingested starch. For comparison, direct measurement of the starch flowing from ileum of healthy ileostomates indicated that 0·03 (freshly-cooked potato) to 0·12 (cooked and cooled potato) of the ingested potato starch escaped small intestinal digestion (Englyst & Cummings, 1987*a*). When mixed diets containing wheat and potato starch were eaten, 0·01–0·05 of ingested starch was recovered in the ileal effluent of healthy ileostomates (Chapman *et al.* 1985).

A greater proportion of the ingested OM flowed from the terminal ileum with the 'instant'-potato diets which suggests that the rehydrated 'instant'-potato contained components which were more resistant to small intestinal digestion or, alternatively, that these diets promoted the flow of additional endogenous OM to the terminal ileum.

Carbohydrate digestion, within the human small intestine, of freshly-prepared instant potato from a different manufacturer (Cadburys Smash®) was similar to that of freshly-cooked potato in a study by Englyst & Cummings (1987*a*). There was no evidence that 'instant' potato undergoes retrogradation more rapidly or extensively than the conventionally-prepared product in the present study (Fig. 1).

LB fermentation and caecal hypertrophy

Despite similar diet compositions, markedly different patterns of caecal fermentation were established with higher pH, lower total concentrations of VFA and longer TT in rats eating the unprocessed potatoes. The larger caecal size, smaller amount of OM fermented daily and much longer TT in the latter rats meant that these animals experienced a much reduced fermentation rate (amount of OM fermented per unit mass of caecal contents per unit time). If caecal steady-state is assumed, then it can be calculated that the caecal VFA pool was turned over 2·4 times as rapidly for diet 4 ('instant'-potato diet fed after storage) as for diet 1 (unprocessed-potato diet fed fresh). There is no obvious reason for these effects on caecal fermentation pattern and other studies in which additional fermentable material was provided have given divergent results. Inclusion of raw peas (*Pisum sativum*) in an otherwise non-starch polysaccharide (NSP)-free diet also reduced caecal TT and, at low pea inclusion levels, caecal mass; but caecal mass increased as greater amounts of peas and, therefore, fermentable material, were eaten (Goodlad & Mathers, 1990). Inclusion of black eye beans (*Vigna unguiculata*) in a white-rice diet let to a doubling of caecal weight but was without effect on caecal TT (Mathers *et al.* 1990). However, all three of these studies were consistent in showing increases in the molar proportion of butyrate in the caecal VFA with diets which supplied greater amounts of fermentable material to the LB. Possible reasons for this shift in fermentation pattern are discussed by Goodlad & Mathers (1990) with particular attention being given to the observations of high butyrate fermentations with starch as substrate in vitro (Englyst *et al.* 1987; Goodlad & Mathers, 1988), in rats fed on amylomaize (a source of starch relatively resistant to pancreatic amylase; Mallett *et al.* 1988) and in human subjects consuming high-starch diets and taking the α-glucosidase (*EC* 3.2.1.20) inhibitor acarbose (Scheppach *et al.* 1988*b*). However, substrate supply may not be the only factor influencing fermentation end-product pattern. Other environmental effects may also exert selective pressure on the LB microbes altering the balance between species (Mathers *et al.* 1990) or between metabolic pathways. In general, higher proportions of butyrate accompany shorter caecal TT (Fig. 2). The values in Fig. 2 suggest that caecal butyrate proportion is particularly sensitive to changes in caecal TT below about 0·75 d and the effects of caecal butyrate proportion of altering TT over the range 0·3–0·75 d deserves further study. Under these circumstances greater butyrate synthesis may be a means for disposing of reducing equivalents to enable glycolysis to proceed rapidly (Leng, 1970; Goodlad & Mathers, 1990).

Rats fed on the unprocessed potatoes had larger caecums with both more tissue and more contents than those eating 'instant' potatoes. It has frequently been reported (for example, see Wyatt *et al.* 1988; Rémésy & Demigné, 1989; Seal & Mathers, 1989) that increased intake of carbohydrates which are not digested in the small intestine results in enlarged caecums. When these carbohydrates are fermented in the LB, VFA are produced and it has been shown that direct infusion of VFA into the distal intestine stimulates gut epithelial proliferation with butyrate provoking more rapid proliferation than propionate or acetate (Sakata, 1987). This led to the suggestion that the VFA, and in particular butyrate, are lumen trophic factors (Sakata, 1987). However, caecal hypertrophy also occurs when non-digestible polysaccharides which are not (or only poorly) fermented are fed (Wyatt *et al.* 1988) and in the germ-free animal (Goodlad *et al.* 1989) in the absence of

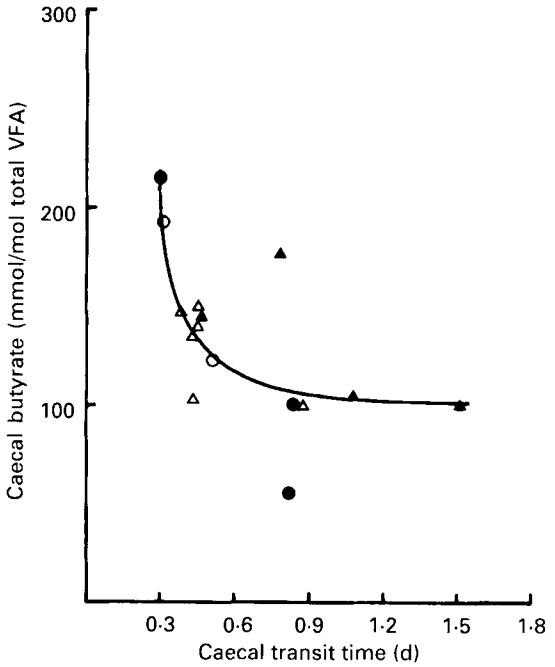

Fig. 2. Relationship between molar proportion of butyrate in volatile fatty acids (VFA) in caecal contents (y) and caecal transit time (x) in rats fed on various diets. Values are taken from (○) Mathers & Fotso Tagny (1989) (using means for each diet), (●) Mathers *et al.* (1990), (△) Goodlad & Mathers (1990) and (▲) the present study. The fitted line is an hyperbola of the form: $y = 94{\cdot}3 - \{36{\cdot}7/(1 - 4{\cdot}35\,x)\}$, which accounted for 0·60 of the variation in y.

fermentation. An alternative hypothesis has been proposed by Wyatt *et al.* (1988) who suggested that LB hypertrophy is 'not primarily a response to bacterial fermentation' but that 'the caecum enlarges to accommodate the tendency of residual material to accumulate within it, and this is governed by the pattern of motility and bulk flow through the whole large bowel'. Our experiment provides no support for Sakata's (1987) hypothesis since more total VFA and more butyrate were produced with the 'instant'-potato diets yet these had smaller caecums. In addition, in our study there was more caecal tissue with the unprocessed-potato diets where there was more 'residual material' (wet caecal contents) in agreement with the observations of Wyatt *et al.* (1988), but it is not at all clear why this greater accumulation of caecal contents should have occurred since there was less material flowing into the LB from the ileum with these diets. Further study of the relationships between substrate flow, the activities of caecal flora and LB motility may be helpful in understanding in which circumstances 'residual material' accumulates in the caecum and how this influences tissue hypertrophy.

Coprophagy

Coprophagy is practised by many animals, especially rodents such as the rat (Hörnicke & Björnhag, 1980), with very variable amounts of faeces apparently being ingested under different circumstances. Measurement of the quantity of faeces ingested has often been made by indirect methods involving comparison of faecal output from animals fitted with tail cups (which prevent re-ingestion) with that from animals allowed to practise coprophagy (Fajardo & Hörnicke, 1989). Such studies have indicated that with

nutritionally-complete diets there is relatively little re-ingestion (0–11%) but this can rise to 25% (Fajardo & Hörnicke, 1989) or more (Hörnicke & Björnhag, 1980) with unbalanced or nutrient-deficient diets. Such re-ingestion of faeces could affect the interpretation of gastrointestinal flow and TT studies where an indigestible marker (such as Cr_2O_3 in the present study) is used. It was important, therefore, to ascertain the extent of coprophagy by our animals and we did this by a simple and apparently novel method, i.e. comparison of the concentration of marker in stomach contents DM with that in food and in faeces. This calculation assumes that food and faeces are the only significant contributors to stomach contents DM. In our study there was no evidence of significant re-ingestion of faeces and so classical marker-ratio methods may be used with confidence, but this may not always be the case and appropriate checks should be made.

Possible implications for tissue metabolism and health

The increased flow of OM to the LB and reduced TT in both the caecum and colon with the 'instant'-potato diets would, if they occurred in man, be beneficial in alleviating constipation. These 'instant'-potato diets were also responsible for the apparent absorption of larger amounts of VFA provided by both acetate and butyrate. Increased butyrate production may be beneficial since it is a preferred energy substrate for the LB mucosa (Roediger, 1980, 1982) and is reported to have anti-neoplastic properties (Cummings & Branch, 1982) but could be contra-indicated in those with compromized livers. The longer-chain VFA, propionate and butyrate, are found in human peripheral blood only in exceptional circumstances usually associated with liver damage (Trauner *et al.* 1975; Lai *et al.* 1977), and the hepatic removal of longer-chain VFA in the healthy individual may be protective since butyrate by-passing the liver into the systemic circulation has narcotic effects (Samson *et al.* 1956; Walker *et al.* 1970) and may be a factor in the aetiology of hepatic encephalopathy (Zieve & Nicoloff, 1975). Increased absorption of acetate is unlikely to have any detrimental effect on tissue metabolism since it is a normal blood metabolite which can be oxidized or used for synthetic purposes by many tissues. Recent studies have shown that additional gut-derived acetate has no significant effect on glucose metabolism in man (Scheppach *et al.* 1988*a*, *c*).

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