

Gastrointestinal responses to oats consumption in young adult and elderly rats: digestion, large bowel fermentation and crypt cell proliferation rates

BY J. C. MATHERS AND JULIA KENNARD

Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU

AND O. F. W. JAMES

Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH

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The present experiment was designed to test the hypothesis that ageing modifies the gastrointestinal responses to a change in diet composition. Rats of the Wag/Rij strain, either young adult (4 months of age) or elderly (27 months of age), were given a basal semi-purified diet or a diet of similar major nutrient composition containing 500 g oatmeal/kg for 17–18 d. Elderly rats digested the dry matter (DM) and organic matter (OM) of both diets less well than did their young adult counterparts, with more of this digestion occurring in the distal intestine. The greater flow of OM to the caecum of oats-fed animals was accompanied by significant reductions in caecal pH and increases in caecal total short-chain fatty acids (SCFA) concentration which appeared to be independent of age. However, young adults responded to feeding on oats by showing a much larger increase in the molar proportion of butyrate (332%) than did elderly animals (79%). Elderly rats had longer duodenal villi than did young adults but effects of age or diet were not detectable at other sites. With both age-groups oats consumption was associated with significant stimulation of crypt cell proliferation rate (CCPR) in the small intestine and caecum, but for the colon there was a significant reduction in CCPR with oats feeding. A reduced ability of the aged large bowel (LB) to produce butyrate may contribute to the prevalence of LB disorders in the elderly.

Ageing: Oats: Large bowel fermentation: Crypt cell proliferation rate: Rats

Ageing is associated with many changes in form and function of the gastrointestinal tract, some of which may have nutritional consequences through effects on the digestive process, on absorption or on nutrient utilization by gut tissues.

Evidence that the loss of digestive or of absorptive capacity during ageing is sufficiently great to cause malnutrition is scanty (Bhanthumnavin & Schuster, 1977; Thompson & Keelan, 1986). There is some information from human studies (Webster & Leeming, 1975) and much, some of it conflicting, from animal experiments (Clarke, 1972, 1977; Meshkinpour *et al.* 1981; Ecknauer *et al.* 1982; Holt *et al.* 1984; Raul *et al.* 1988; Goodlad & Wright, 1990) indicating that the morphology of the small intestine (e.g. villus length and shape, numbers of crypts per villus and surface area available for absorption) alters during ageing, but the significance of these changes for nutrient digestion and absorption is largely unknown. Some studies in animals have suggested that ageing is associated with reductions in glucose absorption (Esposito *et al.* 1985; Freeman & Quamme, 1986) whilst others have found no such effects (Hirst & Wallis, 1990). Similar uncertainty exists in respect of activities of brush-border hydrolases with some authors reporting reductions in activities (Höhn *et al.* 1978; Holt *et al.* 1985) which appeared to be due to an increase in the

proportion of relatively undifferentiated villus epithelial cells (Holt *et al.* 1985), whilst similar (Moog, 1977; Freeman & Quamme, 1986) or increased (Raul *et al.* 1988; Darmenton *et al.* 1989) activities of these enzymes were reported by others. Suggestions that fat absorption may be decreased in the elderly (Webster *et al.* 1977) have not been confirmed in later human and animal experiments (for review, see Thompson & Keelan, 1986) and there is no evidence of impaired vitamin B₁₂ absorption in the normal elderly human (McEvoy *et al.* 1982). Water absorption per unit mass of small intestine was little altered between 8 and 120 weeks of age in male Sprague–Dawley rats (Goodlad & Wright, 1990).

Much of the research on the effects of diet on ageing has been concerned with the prolongation of lifespan by energy intake restriction (Masoro, 1985; Walford *et al.* 1987) and there is evidence that this strategy alters small bowel architecture (Heller *et al.* 1990), but there appears to have been little systematic study of the gastrointestinal responses of aged animals to alterations in diet composition. The objective of the present experiment was to test the hypothesis that ageing modifies the gastrointestinal responses to a change in diet composition, i.e. inclusion of oatmeal in a semi-purified basal diet using rats as the experimental subjects. The non-starch polysaccharides (NSP) in the oatmeal were expected to provide the large bowel (LB) microflora with increased supplies of fermentable substrate. Measurements were made of sites and extent of digestion of dry matter (DM) and organic matter (OM), of caecal fermentation, of gut morphology and of crypt cell proliferation rate (CCPR).

A brief account of part of the present study has been published (Mathers *et al.* 1991).

EXPERIMENTAL

Animals and housing

Twenty-four rats of the Wag/Rij strain (Burek, 1978) were imported from the Eurage facility (TNO Institute for Experimental Gerontology, Huiswijk, The Netherlands). Twelve were young adults (4 months of age, six male and six female) and twelve were elderly (27 months of age, six male and six female). Throughout the study the animals were housed individually in plastic cages with wire-mesh floors in a quarantine area within the Comparative Biology Centre, University of Newcastle upon Tyne.

Diets and feeding

For 13 d before the start of the study the animals were fed *ad lib.* on a stock diet (RMI(E); Special Diet Services Ltd, PO Box 705, Witham, Essex). Two experimental diets were formulated as described in Table 1. The basal diet contained 20 g cellulose/kg as the only source of NSP whilst the other diet contained 500 g medium oatmeal (Mornflake Oats Ltd, Crewe, Cheshire)/kg included at the expense of maize starch, sucrose, casein and maize oil to maintain similar dietary concentrations of starch, protein, and oil. Cr₂O₃ was included as an indigestible flow marker.

Three male and three female animals from each age-group were allocated at random to each diet and offered 15 g air-dry diet at 10.00 hours daily for 17–18 d. For days 10–16 inclusive all uneaten food was collected, dried and weighed and faeces were collected quantitatively.

Collection of blood, tissue and digesta samples

Each animal was injected intraperitoneally with an aqueous solution of vincristine sulphate (1 mg/kg body weight in approximately 0.5 ml saline (9 g NaCl/l) 2 h before collection of tissue samples. Approximately 10 min in advance of sample collection, general anaesthesia was induced by subcutaneous injection of Hipnorm–Midazolam cocktail (1.0 ml/300 g body mass; prepared by the Comparative Biology Centre). Laparotomy was performed

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

	Basal	Oats
Oat meal*	0	500
Maize starch†	500	150
Sucrose	168	98
Casein	180	120
Maize oil	80	60
Vitamin + mineral premix‡	50	50
Cellulose§	20	20
Cr ₂ O ₃	2	2

* Medium oatmeal (Mornflake Oats Ltd, Crewe, Cheshire).

† Cornflour 03403 (Cerestar, Trafford Park, Manchester).

‡ Complete vitamin and mineral mix 82127 (SDS, Witham, Essex).

§ α -Cellulose C8001 (Sigma).

and samples of blood from the portal vein (1.5 ml) and the heart (4 ml) collected into heparinized syringes. The liver was excised, washed in ice-cold saline, blotted dry and weighed before being stored in liquid N₂. The gastrointestinal tract, from the stomach to the rectum, was removed and divided into stomach, small intestine, caecum and colon. All gut sections (except the small intestine) were weighed complete with digesta. Stomach and caecal digesta pH were measured and two samples (approximately 1 g) taken and treated with deproteinizing solution (Mathers *et al.* 1990) in preparation for short-chain fatty acids (SCFA) determination. The remaining digesta from the stomach, caecum and colon was transferred to preweighed tubes and stored frozen. The empty gut sections were washed with saline, blotted dry and weighed.

The small intestine length was measured and two 10 mm lengths excised from each of three sites, i.e. 10, 50 and 80 % of the distance from the pylorus. One of each pair of gut samples was placed in formal saline fixative (100 ml formaldehyde/l saline) in preparation for morphological measurements whilst the other sample was immersed in Carnoy's fluid (ethanol-acetic acid-chloroform; 6:1:3, by vol.) in preparation for CCPR determinations. In addition, two portions (approximately 10 mm square) of stomach, caecal and colonic tissue were each fixed in the same manner. The stomach tissue was taken from the fundic/antral region, and the caecal and colonic tissue samples from about 50 % of the distance along the organs.

Sample processing

For morphological measurements fixative was removed by washing in distilled water and tissues then dehydrated through a series of ethanol solutions, i.e. 700 ml/l, 900 ml/l and absolute ethanol (twice). After two washes in chloroform the fixed tissue was embedded in wax and transverse sections (5 μ m thick) were cut, stained and mounted on microscope slides. Villus heights and crypt depths were measured with an image analyser (IAS 25; Joyce-Loebl Ltd, Gateshead, Tyne and Wear) using the GENIAS 25 program, with the microscope images collected via a Panasonic WV/CD50 camera.

For CCPR determinations samples were fixed in Carnoy's fluid for 2–6 h and then transferred to aqueous ethanol (700 ml/l) for storage. When required, small portions (approximately 1–2 mm³) were taken and treated as described by Goodlad & Wright (1982) to prepare micro-dissected crypts. The number of cells arrested in metaphase per crypt was determined in ten crypts per sample.

SCFA in digesta samples and Cr₂O₃ in diets and freeze-dried digesta and faeces samples were determined as described by Mathers *et al.* (1990).

Calculations and statistical analyses

Apparent digestibilities of DM and OM were calculated by the marker ratio technique and transit time (TT) through the caecum and colon as the amount of Cr_2O_3 recovered in each organ divided by daily flow of Cr_2O_3 through that organ (Goodlad & Mathers, 1990; Mathers & Dawson, 1991). The latter was estimated either as daily Cr_2O_3 intake or faecal recovery. Large bowel TT is the sum of caecal and colonic TT.

Using analysis of variance the effects of age (A), diet (D), sex (S) and all combinations of these factors, e.g. AD or DS (each with 1 df source of variation) were tested against the residual variation with 16 df. Possible linear relationships between variables were examined by linear regression. All computations were carried out using the STATGRAPHICS package (STSC Inc., Rockville, MD, USA) on an IBM PC model 50.

Where single factor effects (e.g. A) are tested the two corresponding means each have twelve replicates, two-factor interactions (e.g. AD) have four means each with six replicates, whilst the three-factor interaction (ADS) has eight means each with three replicates. Effects were considered to be statistically significant when $P < 0.05$ and these are indicated in the tables of results.

RESULTS

Animal well-being

With the exception of one elderly male on the basal diet which lost 40 g body weight, all rats maintained body weight or gained slightly during the balance period of the study (Table 2). Five of the elderly females (three from basal diet group and two from the oats-containing diet group) had blotchy livers and two (both basal diet group) had gut and uterine tumours. The (heavier) male rats ate more diet and had significantly greater faecal DM outputs than the females but there was no consistent effect of age or diet on food intake. The significant AS and DS interactions in food intake are due largely to the low intake of the elderly male referred to previously. Absolute liver weights were lower for females than for males but when expressed as a proportion of body mass, the females had greater values than the males and the young had greater values than the elderly.

Digestion and caecal fermentation

Elderly rats digested both diets less well than did their young adult counterparts and this was observed both when faecal DM output was expressed as a proportion of DM intake (Table 2) and when apparent digestibilities of DM and of OM were calculated by the marker ratio method (Table 3), although the effect was not statistically significant in the latter case.

Apparent digestibilities of both DM and OM estimated at the caecum and colon were significantly lower in elderly rats than in young rats suggesting that more food residues reached the LB in the older animals. As expected, the oats-containing diet was less digestible than the basal diet for both age-groups (Tables 2 and 3). Whilst apparently little net digestion of DM or OM occurred in the LB of the basal diet-fed rats (as indicated by the similar values for apparent digestibility measured at the caecum and colon and in faeces), there was evidence that at least 1–3% additional digestion occurred in the LB of the oats-fed rats.

The greater flows of OM to the caecum of oats-fed animals (Table 3) were accompanied by significant reductions in caecal pH and increases (mean 75%) in caecal SCFA concentration which were apparently independent of age (Table 4). The young adults responded to feeding on oats by showing a very large (mean 332%) increase in the molar proportion of butyrate; the increase seen in the elderly animals was more modest (mean 79%) and the AD interaction was highly significant ($P < 0.01$). These increases in butyrate were at the expense of acetic (young only), propionic, isobutyric, isovaleric and valeric

Table 2. *Body weights at start of balance period, weight changes, food dry matter (DM) intakes, faecal DM outputs and liver weights of male and female, young adult and elderly Wag/Rij rats fed on basal and oats-containing diets†*
(Means for three rats per treatment group)

Age (A) ...	Young				Elderly				Statistical significance of treatment effects‡	
	Basal		Oats		Basal		Oats			
	♂	♀	♂	♀	♂	♀	♂	♀		
Initial weight (g)	324	178	292	182	359	230	393	231	12.4	A*** S***
Weight change (g/7d)	7	1	12	1	-15	7	3	5	5.4	AS*
DM intake (g/7d)	92	64	92	65	62	76	96	74	5.7	S* AS* DS*
Faecal DM output (g/7d)	7.1	4.7	9.7	6.8	5.8	6.2	11.7	9.2	0.65	S* D* AD*
Faecal DM (g/100 g DM intake)	7.7	7.5	10.5	10.5	9.2	8.2	12.2	13.0	0.34	A*** D***
Liver (g)	9.7	5.6	8.7	6.1	8.4	7.0	9.7	7.6	0.52	S***
Liver (g/kg body mass)	29.2	31.4	28.6	33.3	24.3	29.4	24.4	32.1	1.46	A*** S***

* $P < 0.05$, *** $P < 0.001$.

† For details of diets, see Table 1.

‡ For details of statistical analysis, see p. 571.

Table 3. *Apparent digestibilities of dry matter and organic matter, estimated by the marker ratio method, at various intestinal sites in male and female, young adult and elderly Wag/Rij rats fed on basal and oats-containing diets†*

(Means for three rats per treatment group)

Age (A)...	Young				Elderly				SEM	Statistical significance of treatment effects‡
	Basal		Oats		Basal		Oats			
Diet (D)...	♂	♀	♂	♀	♂	♀	♂	♀		
Sex (S)...	♂	♀	♂	♀	♂	♀	♂	♀		
Dry matter										
Caecum	0.95	0.94	0.88	0.90	0.93	0.93	0.86	0.87	0.007	A** D***
Colon	0.95	0.94	0.90	0.91	0.93	0.94	0.88	0.87	0.007	A*** D***
Faeces	0.94	0.94	0.90	0.90	0.93	0.94	0.89	0.88	0.004	D*** DS*
Organic matter										
Caecum	0.97	0.96	0.91	0.92	0.95	0.96	0.90	0.89	0.007	A** D*** ADS*
Colon	0.97	0.96	0.93	0.94	0.96	0.97	0.91	0.91	0.006	A** D***
Faeces	0.96	0.97	0.93	0.93	0.96	0.96	0.92	0.91	0.003	D***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets, see Table 1.

‡ For details of statistical analysis, see p. 571.

Table 4. *Caecal pH, total short-chain fatty acids (SCFA) concentration and molar proportions of individual SCFA in male and female, young adult and elderly Wag/Rij rats fed on basal and oats-containing diets†*

(Means for three rats per treatment group)

Age (A)...	Young				Elderly				SEM	Statistical significance of treatment effects‡
	Basal		Oats		Basal		Oats			
Diet (D)...	♂	♀	♂	♀	♂	♀	♂	♀		
Sex (S)...	♂	♀	♂	♀	♂	♀	♂	♀		
Caecal pH	7.1	7.3	6.5	6.5	7.2	7.1	6.5	6.9	0.15	D***
Total SCFA (mmol/kg caecal contents)	100	72	142	157	65	100	167	124	14.6	D*** ADS**
Molar proportions of individual SCFA (mmol/mol total SCFA)										
Acetate	613	586	495	494	614	628	630	632	39.9	A*
Propionate	189	196	130	111	228	181	147	225	13.8	D*** A*** AD*** DS* ADS**
Isobutyrate	36	66	23	24	35	42	18	18	9.0	D**
Butyrate	104	88	308	330	65	90	170	108	30.8	D*** A*** AD**
Isovalerate	30	38	18	17	38	30	15	14	2.6	D***
Valerate	24	27	18	17	21	30	13	3	3.3	D*** DS*
Hexanoate	5	nd	11	10	nd	nd	5	nd	2.3	D** A**

nd, not detected.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets, see Table 1.

‡ For details of statistical analysis, see p. 571.

Table 5. Stomach tissue and digesta weights, pH and total short-chain fatty acids (SCFA) concentration and small intestinal length in male and female, young adult and elderly Wag/Rij rats fed on basal and oats-containing diets†
(Means for three rats per treatment group)

Age (A) ...	Young				Elderly				Statistical significance of treatment effects‡	
	Basal		Oats		Basal		Oats			
	♂	♀	♂	♀	♂	♀	♂	♀		
Diet (D) ...										
Sex (S) ...										SEM
Stomach										
Whole organ (g)	2.60	1.98	3.09	2.79	3.06	3.24	4.68	3.20	0.409	D* A***
Tissue (g)	1.40	0.99	1.36	1.04	1.63	1.33	1.82	1.42	0.0058	A** S*** ADS**
Tissue (g/kg body mass)	4.2	5.5	4.5	5.7	4.8	5.6	4.6	5.9	0.12	S***
Digesta, wet (g)	1.20	0.98	1.74	1.75	1.43	1.91	2.85	1.78	0.387	D* A*
Digesta, dry (g)	0.35	0.25	0.61	0.79	0.60	0.61	0.93	0.51	0.131	D* A*
pH	2.4	3.1	4.8	4.5	6.0	4.2	4.7	4.8	0.60	A* AD*
Total SCFA (mmol/kg digesta)	12	11	11	24	10	10	14	18	5.0	
Small intestine										
Length (cm)	115	100	109	100	113	110	120	106	2.4	A** S*** ADS*
Length (cm/kg body mass)	348	560	361	546	329	463	302	448	16	A*** S*** AS*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets, see Table 1.

‡ For details of statistical analysis, see p. 571.

Table 6. *Caecal and colonic tissue and digesta weights in male and female, young adult and elderly Wag/Rij rats fed on basal and oats-containing diets†*

(Means for three rats per treatment group)

Age (A)...	Young				Elderly				SEM	Statistical significance of treatment effects‡		
	Basal		Oats		Basal		Oats					
Diet (D)...	♂	♀	♂	♀	♂	♀	♂	♀				
Sex (S)...	♂	♀	♂	♀	♂	♀	♂	♀				
Caecum												
Whole organ (g)	2.74	1.75	3.92	3.02	3.02	2.63	4.33	4.39	0.279	D***	A***	S*
Tissue												
g	0.69	0.75	0.81	0.68	0.81	0.79	1.07	1.02	0.102		A*	
g/kg body mass	2.1	4.2	2.7	3.7	2.4	3.4	2.7	4.3	0.53		S**	
Digesta (g)												
Wet	2.05	1.00	3.11	2.34	2.21	1.84	3.26	3.37	0.283	D**	A*	S*
Dry	0.48	0.25	0.68	0.53	0.50	0.39	0.68	0.57	0.042	D**	S***	
Colon												
Whole organ (g)	2.54	1.38	2.57	1.49	2.03	1.81	3.02	2.13	0.277		S***	
Tissue												
g	1.23	0.79	1.11	0.91	1.25	1.07	1.63	1.20	0.074	D*	A***	S***
g/kg body mass	3.7	4.4	3.6	5.0	3.6	4.5	4.1	5.1	0.21	AD*	ADS*	S***
Digesta (g)												
Wet	1.31	0.59	1.46	0.58	0.77	0.74	1.38	0.98	0.273		S*	
Dry	0.43	0.47	0.65	0.36	0.35	0.28	0.49	0.38	0.125			

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets, see Table 1.

‡ For details of statistical analysis, see p. 571.

acids. Hexanoate was more commonly detected in caecal contents of oats-fed rats and in the young rats compared with the older animals.

Stomach and small intestine

Older rats had significantly heavier stomachs due to increases in the masses of digesta ($P < 0.05$) and of tissue ($P < 0.01$) and similar effects were seen when comparing oats-fed and basal diet-fed animals (Table 5). Female rats had less absolute weight of stomach tissue but the latter formed a significantly greater proportion of body mass for females than it did for males ($P < 0.001$). Stomach pH was significantly increased in elderly *v.* young rats with the effect being more pronounced for the basal than for the oats-containing diet. SCFA were detected (overall mean 14 (SE 2.5) mmol/kg digesta) in stomach contents with no apparent effects of A, D or S.

The small intestine was longer in elderly rats than in young adult animals and in males than in females. However, whilst relative small intestinal length (mm/kg body mass) was less in the elderly than in younger adults, on this basis it was considerably greater for females than for males. The significant ($P < 0.05$) AS interaction highlights the more pronounced effect of gender in the younger animals.

Large bowel

Caecal weights were significantly greater for oats-fed rats than for basal diet-fed rats, for elderly than for young adults, and for male than for female animals (Table 6). These differences were due largely to greater contents of wet digesta but there was also a

Table 7. Cr_2O_3 in the caecum and colon and transit times (TT) of this marker in the large bowel of male and female, young adult and elderly Wag/Rij rats fed on basal and oats-containing diets[‡]

(Means for three rats per treatment group)

Age (A)...	Young				Elderly				SEM	Statistical significance of treatment effects [§]
	Basal		Oats		Basal		Oats			
Diet (D)...	♂	♀	♂	♀	♂	♀	♂	♀		
Caecum										
Cr_2O_3 mg/g digesta DM	31.3	29.0	17.7	20.2	23.5	25.7	15.1	15.3	1.95	A** D***
mg/organ	14.9	7.4	12.1	10.7	11.9	9.7	10.2	8.6	1.45	S*
TT (d)										
a	0.71	0.49	0.45	0.56	0.97	0.54	0.37	0.41	0.157	
b	0.61	0.54	0.44	0.57	0.74	0.41	0.34	0.41	0.132	
Colon										
Cr_2O_3 mg/g digesta DM	33.2	28.6	20.4	22.3	25.0	26.6	17.2	15.7	1.64	A*** D***
mg/organ	14.3	5.3	13.4	8.0	9.2	7.6	8.2	6.3	3.19	
TT (d)										
a	0.65	0.35	0.50	0.42	0.58	0.42	0.29	0.27	0.143	
b	0.51	0.31	0.50	0.39	0.44	0.34	0.27	0.33	0.127	
Large bowel										
TT (d)										
a	1.29	0.84	0.95	0.98	1.55	1.01	0.66	0.66	0.223	D†
b	1.09	0.66	0.94	0.92	1.18	0.79	0.61	0.74	0.191	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† $P = 0.057$.

‡ For details of diets, see Table 1.

§ For details of statistical analysis, see p. 571.

|| TT was calculated as described on p. 571 using (a) dietary intake and (b) faecal recovery of Cr_2O_3 to estimate daily flow of Cr_2O_3 through the organ.

significantly greater mass of caecal tissue in older than in younger adult rats. When tissue masses were expressed as a proportion of body weight, between D and between A effects were no longer apparent but values for females were significantly greater than for males. The caeca of oats-fed rats contained more DM than did those of basal diet-fed animals, with males containing more digesta DM than females at both ages.

Colons for male rats were heavier than those from females with, on average, 31% more tissue and 70% more wet digesta contents (Table 6). Relative colonic tissue mass (g/kg body mass) was very significantly greater for females than for males and 10% greater for oats-fed rats than for basal diet-fed rats. The older group of rats contained, on average, 27% more colonic tissue than the younger group.

Estimation of transit time (TT) through the caecum and colon was based on the recovery of the non-absorbable marker, Cr_2O_3 , in the organ relative to daily intake. The lower digestibilities observed with the oats-containing diet and in the older rats are reflected in the lower concentrations of Cr_2O_3 in caecal and colonic digesta DM (Table 7), but when the greater masses of caecal DM for these treatment groups (Table 6) were taken into account, there were no statistically significant effects of A or D on total Cr_2O_3 in the caecum or in

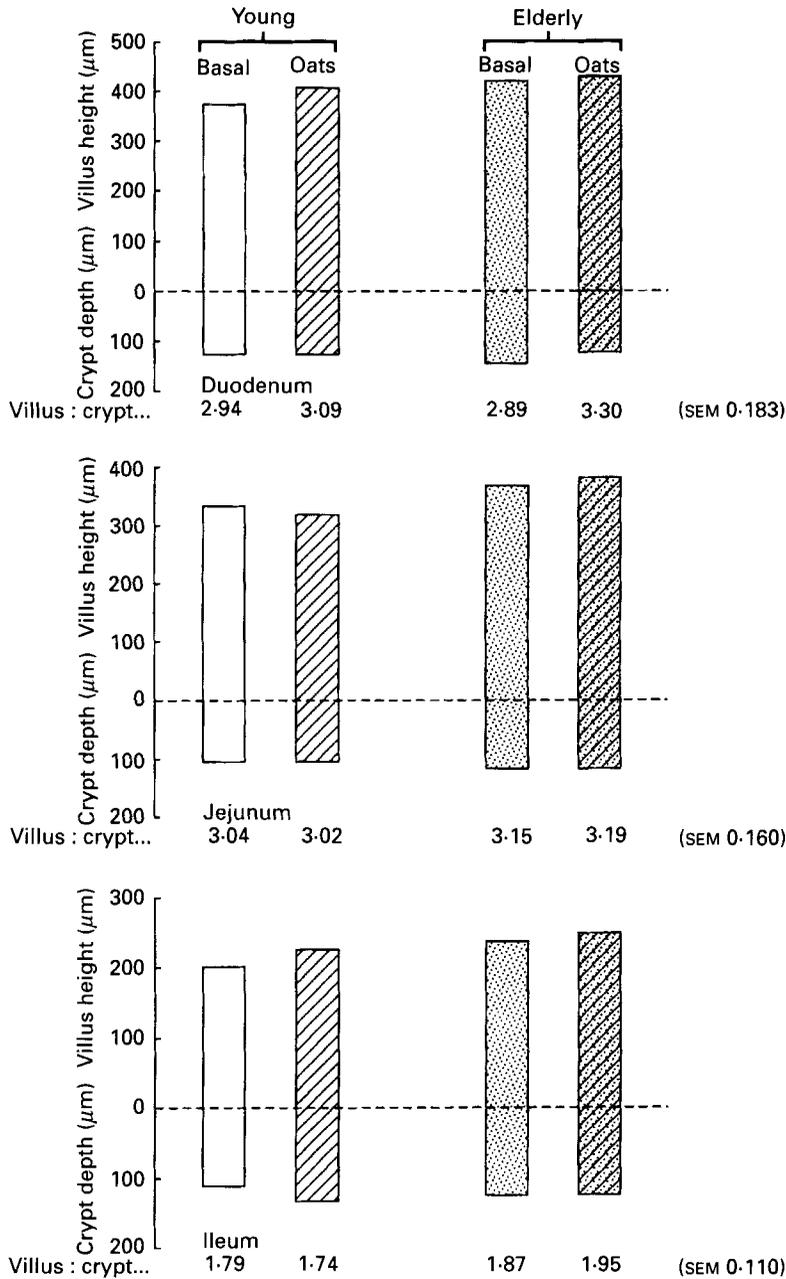


Fig. 1. Villus heights and crypt depths (μm) and villus height: crypt depth for small intestinal tissue samples removed from 10 (duodenum), 50 (jejunum) and 80 (terminal ileum) % of the distance from the pylorus of young adult (\square \boxtimes) and elderly (\boxtimes \boxtimes), Wag/Rij rats fed on basal (\square \boxtimes) and oats-containing (\boxtimes \boxtimes) diets (for details of diets, see Table 1). (---). Crypt-villus junction. Values are means for six rats per treatment group; SEM for duodenum, jejunum and ileum villus heights and crypt depths were 26.2 and 5.6, 17.2 and 4.0, and 16.9 and 6.9 μm respectively. Duodenal villi were significantly ($P = 0.007$) longer in elderly animals.

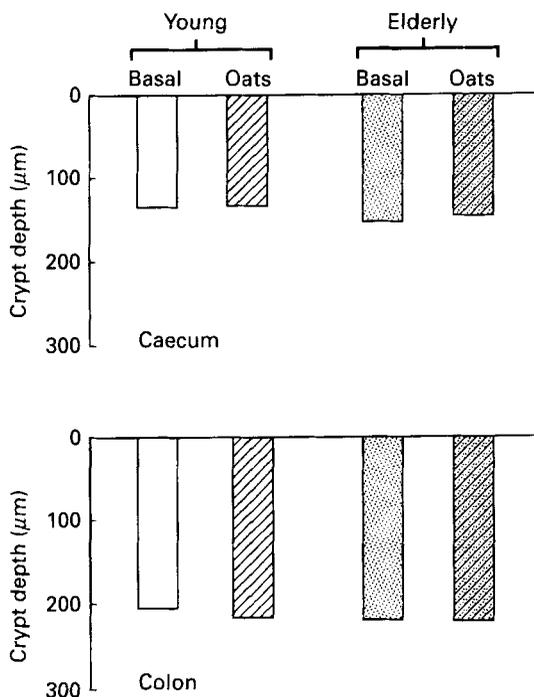


Fig. 2. Crypt depths (μm) for caecal and colonic tissue of young adult (\square \square) and elderly (\square \square) Wag/Rij rats fed on basal (\square \square) and oats-containing (\square \square) diets (for details of diets, see Table 1). Values are means for six rats per treatment group; SEM for caecal and colonic crypt depths were 5.9 and 11.6 μm respectively. Caecal crypts were significantly ($P = 0.024$) deeper in elderly animals.

the colon (Table 7). In consequence, there were no significant effects of A, D or S on TT for either compartment of the LB. There was a tendency for TT to be lower in the oats-fed animals which almost reached statistical significance ($P = 0.057$) when TT in the whole LB (caecum + colon) was determined. Whilst there was some variation in the values of TT obtained for the basal diet groups depending on whether dietary intake or faecal recovery of Cr_2O_3 was used to estimate daily flow of Cr_2O_3 through the organ, values for the oats-fed group were relatively independent of the method of calculation and both procedures produced similar conclusions as to treatment effects.

Gastrointestinal morphology and epithelial proliferation rate

For all animals, villus heights were greatest for duodenal tissue and declined to the terminal ileum (Fig. 1). Elderly rats had longer duodenal villi than their younger counterparts but effects of A or D were not detectable at other sites. Small intestinal crypt depths appeared to be less at the 50% than at the 10 or 80% sites but treatment effects were not statistically significant. Overall values for villus length: crypt depth were markedly lower for the terminal ileum than for the more proximal sites but no treatment effects were detected.

Colonic crypts were considerably deeper than caecal crypts for all groups and the younger rats had significantly shallower crypts than the older ones (Fig. 2).

Intestinal site and diet were the major factors influencing the rate of CCPR. Within the small bowel, CCPR on the basal diet was similar at all three sites and increased on the oats-containing diet for both young adult and elderly groups (Fig. 3). For the jejunum only, elderly rats had higher CCPR. The terminal ileal CCPR of young animals appeared to respond more markedly to oats consumption than did that of the elderly rats, resulting in

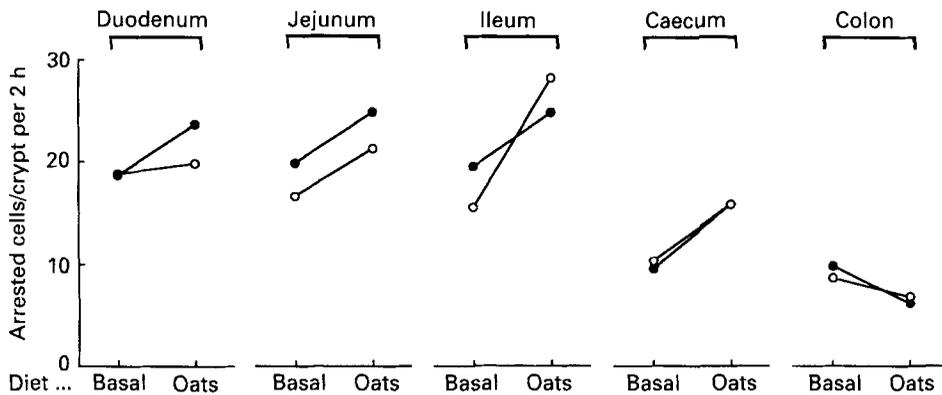


Fig. 3. Crypt cell proliferation rates (CCPR), estimated as the number of cells per crypt arrested in metaphase during 2 h after vincristine injection, in samples removed from 10 (duodenum), 50 (jejunum) and 80 (terminal ileum) % of the distance from the pylorus, and from the caecum and colon of young adult (O-O) and elderly (●-●) Wag/Rij rats fed on basal diets and oats-containing diets (for details of diets, see Table 1). Values are means for six rats per treatment group; SEM for duodenum, jejunum, ileum, caecum and colon were 1.34, 0.98, 1.20, 0.57 and 0.53 respectively. Effects of diet were significant ($P < 0.01$) at all intestinal sites with the elderly rats having significantly ($P = 0.016$) higher CCPR than the young in the jejunum. The age \times diet effect was significant ($P < 0.05$) for ileal tissue.

a significant AD interaction. Caecal CCPR on the basal diet was only about half that of the corresponding rate in the small intestine, was very similar for young and elderly animals, and increased by approximately 60% with oats consumption for both age-groups. In contrast, feeding oats was associated with a significant reduction of CCPR within the colon.

DISCUSSION

Sites and extent of digestion

Our elderly rats digested the DM and OM of their diets significantly less effectively than did the young adults but the decrease of 1–2 percentage units is unlikely to have much nutritional importance. Of more interest is the apparent distal shift in the site of digestion so that a greater proportion of digestion occurred in the LB of elderly rats than in that of young rats. Breath H_2 responses in healthy aged human volunteers to carbohydrate-rich test meals suggested carbohydrate malabsorption in the absence of evidence of malnutrition (Feibusch & Holt, 1982). We have no information on the relative importance of the small bowel compartments (duodenum, jejunum and ileum) between the age-groups in our study but Heller *et al.* (1990) noted that ileal villus cellularity increased throughout the lifespan of *ad lib.*-fed rats, possibly because of a gradually increasing lumen nutrient load. Restricting intake to 60% of the *ad lib.* rates, which would be expected to prevent overloading of proximal digestive capacity, reduced ileal cell number.

The importance of the LB in energy salvage is indicated by the improvements in digestion between the caecum and faeces for the oats-fed animals. This probably underestimates the true extent of LB digestion which is better determined as the difference in flow between the terminal ileum and output in faeces (Goodlad & Mathers, 1990; Mathers & Dawson, 1991), but this information was not available for the current study.

Fermentation in the large bowel

The additional supply of fermentable OM to the LB with oats-feeding resulted in the expected increase in caecal total SCFA concentration and fall in pH (Goodlad & Mathers,

1990; Mathers *et al.* 1990; Mathers & Dawson, 1991) with no detectable effect of ageing. However, old age was associated with a severely blunted response in caecal butyrate to oats consumption. The absolute concentration of butyrate increased 5.7-fold from 8.4 to 47.5 mM in the young adults but only 2.8-fold from 6.6 to 18.4 mM (SE 2.36) in the elderly animals. The reason for this smaller increase in caecal butyrate is not clear. Increased caecal butyrate has been associated with greater flows of fermentable material to the LB (Cheng *et al.* 1987; Tulung *et al.* 1987; Walter *et al.* 1988; Goodlad & Mathers, 1990) particularly when starch is the fermentation substrate (Macfarlane & Englyst, 1986; Goodlad & Mathers, 1988; Mallett *et al.* 1988; Scheppach *et al.* 1988). However, the available evidence suggests that more, not less, OM was fermented in the LB of the elderly animals (Table 3). Based on studies with young rats, Mathers & Dawson (1991) suggested that caecal TT may have an important influence on the proportion of butyrate in fermentation end-products with butyrate increasing sharply when caecal TT was reduced below 0.75 d. In the present study caecal TT did not differ significantly between age-groups; indeed, TT tended to be shortest in the older rats on the oats-containing diet (Table 7). These results highlight the current ignorance of the factors controlling patterns of SCFA production in the LB. Alterations in the relative proportions of major bacterial species (Mathers *et al.* 1990), in environmental conditions, e.g. pH and TT (Sillely & Armstrong, 1984; Finlayson, 1986), in substrate supply (Englyst *et al.* 1987; Goodlad & Mathers, 1988) and in route of disposal of H₂ (Gibson *et al.* 1990) may all influence SCFA pattern. An increased molar proportion of butyrate may also result from more interconversion of acetate to butyrate, as is clearly established from work with ruminant animals (Leng, 1970) and rabbits (Parker, 1976; Woodnutt, 1984) and has recently been observed in *in vitro* incubation with human colonic flora (J. C. Mathers, unpublished results). Since pyruvate is the common intermediate in production of all SCFA, the need is to identify the key factors influencing the flow of C from pyruvate onwards and to clarify how these are regulated by exogenous and endogenous effectors.

An alternative possibility is that the production of butyrate in elderly animals was not reduced when compared with the young but that there was faster absorption of butyrate in the older animals. Since Thompson (1980) found that *in vitro* uptake of saturated fatty acids including SCFA by jejunal tissue was lower for old (11–12 months) than for mature (6–8 weeks) New Zealand albino rabbits, this possibility seems unlikely. It may be argued that Thompson's (1980) evidence is inconclusive since it was obtained with small intestinal tissue from rabbits whilst the LB is the major site of SCFA absorption in non-ruminants. However, the proximal intestine of several species, including man (Schmitt *et al.* 1976) and the rat (Watson *et al.* 1990), readily absorbs SCFA and there is no *a priori* reason to expect that age effects on this process in the rabbit would be different from those in other mammals. Until appropriate direct experimental evidence is available it must remain open to question whether ageing affects absorption of individual SCFA differentially.

Mucosal histology and crypt cell proliferation rate

Villus height and crypt depth are indicators of the size of the absorptive and proliferative compartments respectively in the gut mucosa. Longer villi may be associated with more mature enterocytes exhibiting higher activities of brush-border hydrolases, as is seen in germ-free (Szabo, 1979) or antibiotic-treated (Collington *et al.* 1990) animals. Only for the duodenum of our rats was villus length longer in aged than in young rats (Fig. 1). Holt *et al.* (1984) reported that 27-month-old male Fischer rats had longer ileal villi than their 4–5-month-old counterparts but differences for duodenal and jejunal tissue were not significant. There have been reports that ageing rats have deeper crypts (Holt *et al.* 1984; Holt & Yeh, 1989) than young adults of the same strain kept under similar conditions but other studies

have found no such differences (Clarke, 1977; Ecknauer *et al.* 1982; Goodlad & Wright, 1990). We were unable to detect any difference between age-groups in crypt depth for any intestinal site (Fig. 1). Goodlad & Wright (1990) have cautioned against exclusive reliance on morphological measures such as villus height or crypt depth in studies of the effects of ageing. A better estimate of proliferation is the measurement of vincristine-arrested cells in microdissected crypts (Goodlad & Wright, 1982) and for this variable there was a consistently higher rate for jejunal tissue but not any other site in our elderly rats. Holt & Yeh (1989) reported higher CCPR for jejunum and ileum but similar values for the duodenum for ageing (26–28 months) compared with young (3–4 months) Fischer 344 rats. Since the presence of nutrients in the gut lumen triggers increased CCPR, it is possible that the distal shift in site of digestion in the elderly animals (Table 3) was responsible for the observed increase in jejunal CCPR (present study) and jejunal and ileal CCPR (Holt & Yeh, 1989). In contrast, Goodlad & Wright (1990) found no consistent changes throughout adulthood in CCPR in the proximal and distal small intestine or colon of Sprague–Dawley rats. Differences in the amounts or composition of the diets used may have contributed to these apparent inter-study discrepancies.

Inclusion of oats in the diet was accompanied by significant increases in CCPR throughout the small intestine and in the caecum with young and elderly animals showing similar responses. We are unaware of any other studies which have compared responses in CCPR to changes in diet composition in young adult and elderly animals. In Holt & Yeh's (1989) experiment fasting for 3 d reduced CCPR to a greater extent in young than in ageing rats. Both age-groups responded similarly by returning CCPR to normal values after 24 h refeeding. It is important to distinguish between effects due to re-feeding and those due to changes in diet composition. The experimental diets used in the present study were designed to provide similar amounts of energy but the substitution of oats for, mainly, maize starch was expected to alter the rate and, perhaps, site of starch digestion and to deliver more fermentable substrate to the LB. Provision of additional fermentable substrate by intra-caecal infusion of glucose increased CCPR throughout the small intestine and in the colon of rats (Miazza *et al.* 1985). Similar effects followed the consumption of fermentable NSP-rich diets (Goodlad *et al.* 1987; Johnson *et al.* 1988) or the ileal injection of SCFA (Sakata, 1987) leading to the suggestion that it is the formation of SCFA by bacterial fermentation which is the stimulus for increased CCPR. How this putative stimulus is signalled to proximal enterocytes remains uncertain. Gastrointestinal peptide hormones such as enteroglucagon may play a role (Goodlad *et al.* 1987) but it appears that raised enteroglucagon level alone is not sufficient to increase CCPR (Johnson *et al.* 1988).

There may be direct effects of SCFA on growth of the gut tissue since addition of SCFA to a total parenteral nutrition (TPN) solution reduced the mucosal atrophy associated with TPN after massive bowel resection (Koruda *et al.* 1988). When oat bran (200 g/kg diet) was added to a 'fibre-free' semi-purified diet fed to Sprague–Dawley rats, total colonic crypt labelling index (determined by [6-³H]thymidine incorporation *in vivo*) was unaltered but the labelling index within the proliferative zone increased (Jacobs & Lupton, 1984). This effect also occurred with guar gum but not with pectin. Total number of cells per crypt in the proximal colon was reduced by feeding oat-bran and Jacobs & Lupton (1984) calculated that this diet reduced the time taken for labelled cells to reach the top of the crypt to about half control values. In contrast, in the distal colon all 'fibre' sources increased crypt cell migration times.

The reduction in colonic CCPR with oats consumption in the present study was unexpected and highlights the uncertainty about the hierarchy of factors which control mucosal proliferation. Clearly factors which enhance proliferation at proximal sites may not be present in the colon but the down-regulation of CCPR in the colon of oats-fed

animals compared with controls suggests a more fundamental cause. Recently, Bianchini *et al.* (1992) reported that rectal proliferation was significantly reduced in rats fed on a maize-starch-based diet compared with those given a high-sucrose diet and argued that this may be due to the enhanced caecal and faecal butyrate observed with the starch-rich diet. The apparent contrast between our results and those of Jacobs & Lupton (1984) may be due to our use of oatmeal rather than oat bran. The former is rich in starch, some of which may have reached the caecum to stimulate butyrate production. Jacobs & Lupton (1984) do not present any values for caecal SCFA. Our colonic tissue was taken from about the midpoint of the organ whilst Jacobs & Lupton (1984) examined the proximal and distal colonic segments separately. Differential responses within the colon were reported by Edwards *et al.* (1992) who found that feeding a 'higher-fibre' diet (133 v. 17 g NSP/kg) to male Wistar rats for 18 months, which resulted in increased caecal butyrate, was associated with a significant increase in CCPR in the proximal colon but had no effect in the distal colon. These results appear to contradict the findings by Sakata (1987) of CCPR stimulation in the distal colon of rats following butyrate injection into the caecum.

From a series of studies in pigs fed on different amounts and types of complex carbohydrate, Fleming *et al.* (1992) concluded that it was not possible to predict from markers of LB fermentation the effects of dietary carbohydrates on intestinal proliferation. There is no doubt that the amount and composition of diet can have a major influence on mucosal proliferation at many sites in the gastrointestinal tract but neither the mechanisms involved nor the health consequences are yet apparent.

Conclusions

The contribution of impaired digestion or absorption to malnutrition in old age has not been clearly established. There appears to be an age-related decline in the digestive capacity of the small intestine in man (Feibusch & Holt, 1982) and in rats (Table 3) and there is evidence that, while passive absorption of sugars is unaltered, active absorption is reduced in old age (Beaumont *et al.* 1987). The quantitative significance of these changes is unknown but may be relatively minor since carbohydrate escaping from the small intestine into the LB is subject to bacterial fermentation and energy salvage in the form of absorbed SCFA. Approximately half the carbohydrate energy may be recovered by this means (Livesey, 1990; Mathers, 1991).

Malnutrition among the elderly is more likely to be due to inadequate intake (Thompson & Keelan, 1986) or to concurrent disease (Campbell *et al.* 1989). In man there appears to be a decline in gastric acid output with ageing (Bhanthumnavin & Schuster, 1977; Thompson & Keelan, 1986) and an increase in atrophic gastritis (Bird *et al.* 1977) which may contribute to the greater prevalence of microbial contamination of the small bowel (McEvoy *et al.* 1983; Beaumont & James, 1986), possibly causing malabsorption (Haboui *et al.* 1988). The presence of large populations of microbes in the small intestine may reduce activities of mucosal digestive hydrolases and cause blunting of the villi (Yolton & Savage, 1976; Belosevic *et al.* 1989; Collington *et al.* 1990; Haboui *et al.* 1991) but a recent study has failed to detect any effect of small bowel bacterial contamination on activities of mucosal hydrolases or on glucose absorption by duodenal enterocytes from middle-aged or elderly human subjects (Wallis *et al.* 1993).

To our knowledge this is the first study to compare the LB fermentation response to dietary change in young and aged animals. The apparently reduced production of butyrate by the older animals is of potential significance. Butyrate is an important metabolic fuel for colonocytes (Roediger, 1982) and a relative deficiency of butyrate may contribute to several bowel disorders including starvation diarrhoea (Roediger, 1986) and ulcerative colitis (Breuer *et al.* 1991; Scheppach *et al.* 1992). For reasons which are still unclear, but may

relate to the extent and pattern of acetylation of histones (Kruh, 1982; Smith, 1986), butyrate has antineoplastic properties (Augeron & Laboisse, 1984) so that reduced production of butyrate may predispose to colonic cancer (Bingham, 1990). Given the apparent beneficial effects of butyrate (Cummings & Englyst, 1987), it will be important to discover whether butyrate production is reduced in aged man as it seems to be in rats.

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