The influence of dietary fibre on caecal metabolism in the rat

BY LINDA F. MCKAY AND MARTIN A. EASTWOOD

Wolfson Gastrointestinal Laboratories, Department of Medicine,
Western General Hospital, Crewe Road South, Edinburgh EH4 2XU

1. The influence of three diets ((1) high-fibre – low-fat, (2) low-fibre – high-fat, (3) commercial breeding diet) on the concentration of short chain fatty acids (SCFA) has been investigated in male and female rats up to 14 weeks of age.

2. Hydrogen was detected in respired gas in all rats with no significant differences between diets or sex. Methane was detected only in control-fed rats at 12 weeks of age and thereafter. Caecal contents contained higher concentrations of SCFA than were found in faeces. There were no significant differences in the total faecal SCFA excreted by rats on the three diets. The proportions of SCFA in the caecum and faeces were influenced by diet.

3. The production and excretion of hydrogen, methane and SCFA in the rat appeared to reflect different aspects of bacterial metabolism. Comparison of caecal and faecal SCFA may indicate differential absorption from the colon or differential metabolism by bacteria or colonic mucosa which may be influenced by diet.

The fermentation of dietary fibre by bacteria takes place in the rumen or the large intestine of single-stomached animals. These processes yield carbon dioxide, hydrogen, methane and short chain fatty acids (SCFA) (Hobson, 1972; Hungate, 1976; Bauchop, 1977; McBee, 1977). These biochemical processes have been extensively studied in ruminants and are influenced by food intake and digestibility (Hungate, 1968, 1976). Methane production has been studied in ruminants (Clapperton & Czerkawski, 1969; Czerkawski, 1969; Bryant & Wolin, 1975; Murray *et al.* 1978; Prins, 1979) and in vitro (Stadtman, 1967; Wolfe, 1971) where the formation of hydrogen, methane and SCFA are interrelated and depend on the amount of fermentable substrate present (Czerkawski & Breckenridge, 1969; Prins, 1977).

In contrast to ruminants, bacterial metabolism in the large intestine of single-stomached animals is less well understood (McBee, 1977; Stevens, 1978). The digestive physiology of the pig has been investigated (Cranwell 1968; Kidder & Manners, 1978) but intestinal tract fermentation in laboratory rodents has received little study. SCFA production in the rabbit has been investigated (Henning & Hird, 1972; Parker, 1976; Parker & McMillan, 1976). The absorption of SCFA by the caecal wall in the rat has been demonstrated by Yang *et al.* (1970). In the rat the development of the caecum and its microflora vary according to diet (Remesy & Demigne, 1976) and the influence of diet on hydrogen production in the rat has been reported (Gumbmann & Williams, 1971). However, there are no reports of dietary factors influencing methane production in the rodent.

In other studies (L. F. McKay and M. A. Eastwood, unpublished results) we have shown that fermentation of faeces from herbivorous animals produces methane, whereas fermentation of faeces from carnivores does not. There are also differences in faecal SCFA between the two animal groups.

The present study was designed to investigate the effect of diet on bacterial metabolism in the rat caecum. Rats were given a high-fibre herbivorous diet and a low-fibre carnivorous diet from weaning. Measurements have been made of excreted hydrogen and methane along with SCFA concentration of faeces and caecum contents.

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

Diet...	1 Bran*	2 Meat†	3 Control‡
Carbohydrate	310	—	480
Fat	18	128	34
Crude protein (nitrogen × 6·25)	178	134	213
Dietary fibre	177	—	22
Energy (kJ/kg)	9150	7380	14339

* Equal weights of bran and Spratt's (Barking, Essex) small animal diet.
† Equal weights of raw minced meat and chopped boiled egg.
‡ Spratt's small amimal diet.

METHODS

Rats used in this study were male and female weanling (3 weeks) Wistar rats obtained from a colony of inbred rats maintained in the Animal Unit, Teaching and Research Centre, Western General Hospital. These animals were kept in individual cages on a 12 h light–12 h dark cycle with constant recirculation of filtered air (five to six times/h) and the temperature was maintained at 20–22°.

Eighteen rats were divided into three groups consisting of three males and three females which were given one of three diets from weaning (3 weeks) until 14 weeks of age. Details of diet composition are given in Table 1. Diet 1 (bran diet) (high in fibre and carbohydrate and low in fat) was designed to be a herbivorous type of diet which consisted of equal weights of fine bran and powdered Spratt's laboratory small animal diet (autoclaved), formally known as Spillers' laboratory small animal diet (Spratt, Barking, Essex). Diet 2 (meat diet) contained equal weights of raw minced meat and chopped boiled egg and was a low-fibre, high-fat carnivorous diet. The control diet was Spratt's small animal diet which contains protein from fish, yeast and plant sources and carbohydrate from fish and yeast (diet analysis from K & K-Greeff Chemicals Ltd, Croydon). All diets were fed *ad lib.* and the daily intakes were measured. Water intake was unrestricted.

Body-weights were measured weekly. Production of hydrogen and methane was measured weekly using a closed-circuit apparatus, a modification of the method used by Gumbmann & Williams (1971). Gas production was estimated from the change in gas composition of the closed chamber containing a single animal for 15 min. A 40 ml gas sample was then withdrawn. Based on the sensitivity of the katharometer detector and the volume of the chamber (12 l) the lower limit of detection was 0·09 μmol/l (0·002 ml/l) which required a production rate of 0·094 ml/h. The excretion rate was calculated from the concentration of test gas in the chamber, times the volume of the chamber minus the volume of the rat (density assumed to be 1·00). The ability of the chamber to retain hydrogen and methane without loss was verified by flushing the chamber with 4·5 μmol (0·1 ml) test gas mixture per l for 15 min until full and then sealing the lid. There was no loss of either gas over 105 min. The mean percentage loss after 24 h over five trials was 0·5 methane and 5·4 hydrogen. Hydrogen and methane concentrations were measured by gas–solid chromatography (Tadesse *et al.* 1979) and production was expressed as ml/h per kg body-weight.

Faecal collections (24 h) were obtained from the twelve animals to be fed on the bran and meat diets at 3 weeks and from all animals at 14 weeks. Each animal's faeces were weighed, pooled and freeze-dried. Approximately 100 mg of the resultant powder was analysed for SCFA (Spiller *et al.* 1980).

At the end of the 14-week period (in the afternoon) the rats were killed by a combination

Table 2. *Faecal short chain fatty acids (SCFA) in male and female weanling (3-week-old) rats*

(Mean values and standard deviations for six rats per group)

SCFA (μmol/g)	♂		♀		Statistical significance of difference
	Mean	SD	Mean	SD	
Acetate	32·3	7·5	34	4·16	NS
Propionate	1·62	1·62	4·86	2·56	$P < 0.025$
Butyrate	0·68	0·79	3·18	2·27	$P < 0.025$
Total SCFA (mg/g)	2·12	0·46	2·87	0·98	NS
Faecal wt (g/24 h)	0·485	0·115	0·564	0·104	NS

NS, not significant.

of diethyl ether anaesthesia and cervical dislocation. Before the 14-week period was completed two of the rats (one male, one female) on the meat diet died. Pathological and microbiological analysis were negative. Caecal contents were removed, weighed and adjusted to pH 8·0 before being deep-frozen. Faeces and caecal contents were freeze-dried before SCFA analysis.

Results were analysed using mean values and standard deviations by Student's t test.

RESULTS

Animals gained weight steadily on both test diets with no significant differences from the control diet. The mean (and SD) weights (g) at 12 weeks were 297 (86), 279 (75) and 283 (25) for the bran, meat and control diets respectively.

Daily food consumption reached a mean of 30, 40 and 16 g at 12 weeks for the bran, meat and control diets respectively. This resulted in approximately isoenergetic daily food consumption (274·5, 295·2 and 278·0 kJ respectively) and protein intakes of 5·34, 5·36 and 3·40 g/d respectively.

Hydrogen was detected from all rats at 3 weeks and thereafter (0·63–1·88 ml/h per kg body-weight) with no significant differences either between the diets or between males and females.

Methane was not detected at any time from rats in the two test groups receiving bran and meat diets. Measurable quantities of methane were detected (> 0.094 ml/h per rat) at 12 weeks and thereafter in rats given the control diet, with no significant differences between males and females (means and SD 0·73 (0·152) and 0·591 (0·132) ml/h per kg respectively). Details of faecal weight and faecal SCFA concentration in weanling rats are shown in Table 2. In 3-week-old weanling rats females had significantly more faecal propionate ($P < 0.025$) and butyrate ($P < 0.025$) than male rats. This difference was no longer apparent in 14-week-old rats.

Table 3 shows faecal SCFA concentrations in weanling rats and faecal and caecal SCFA concentrations at 14 weeks on the bran, meat and control diets. Mean (and SD) faecal weights of rats given the bran, meat and control diets were 25·5 (6·9), 3·1 (0·3) and 9·3 (2·5) g/24 h respectively.

Faecal acetate was significantly lower in meat-fed rats ($P < 0.05$) than controls. Faecal propionate, isobutyrate and butyrate were significantly higher ($P < 0.01$, $P < 0.01$ and $P < 0.05$ respectively) in meat-fed rats compared with rats given the control diet. In rats given the bran diet, faecal butyrate concentrations were higher ($P < 0.001$) than in control-fed rats.

Table 3. *Details of faecal and caecal short chain fatty acid concentration ($\mu mol/g$ dry weight) in weanling (3-week-old) rats and at 14 weeks on the bran, meat and control diets**

(Mean values and standard deviations; values in parentheses are molar proportions (mmol/mol))

Group...	Weanling (n 12)		Bran (n 6)					
	Faeces		Faeces			Caecum		
	Mean	SD	Mean		SD	Mean		SD
Acetate	33 (860)	5·8	87 (780)		12·6	235 (590)		50·5
Propionate	3·24 (90)	2·16	9·59 (90)		3·37	43·78*** (110)		5·81
Isobutyrate	0·00 (0)	0·00	0·11 (0)		0·34	0·45*** (0)		0·45
Butyrate	1·93 (50)	1·59	14·54*** (130)		3·75	114·43 (290)		21·93
Isovalerate	0·00 (0)	0·00	0·00 (0)		0·00	1·07 (0)		0·58
Valerate	0·00 (0)	0·00	0·00 (0)		0·00	3·13 (10)		1·86
Dry wt of total SCFA wt (mg/g)	2·50	0·72	7·23		1·18	23·50		9·93

Group...	Meat (n 4)				Control (n 6)			
	Faeces		Caecum		Faeces		Caecum	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Acetate	70* (678)	10·8	138** (581)	12·5	97 (850)	21·6	209 (550)	40·5
Propionate	19·18** (186)	5·40	63·51 (268)	10·40	9·86 (90)	3·64	65·13 (170)	8·78
Isobutyrate	2·04** (20)	0·11	2·15 (9)	0·00	0·56 (0)	0·90	2·5 (10)	1·13
Butyrate	9·43* (91)	2·61	26·93** (113)	9·09	5·00 (40)	3·97	94·77 (250)	21·93
Isovalerate	1·27 (12·5)	1·27	2·74*** (12)	0·00	0·88 (10)	1·17	1·17 (10)	1·07
Valerate	1·27 (12·5)	1·27	4·11 (17)	3·23	1·07 (10)	1·07	5·19 (10)	2·15
Dry wt of total SCFA wt (mg/g)	6·99	1·48	16·28**	2·90	7·16	2·63	27·42	5·60

Mean values were significantly different from control values: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
* For details of animals and diets, see p. 680 and Table 1.

The concentrations of all SCFA were higher in caecal contents than in the faeces. Caecal contents of rats given the bran diet had significantly less propionate ($P < 0.001$) and isobutyrate ($P < 0.001$) than those of control-fed rats. The caecal contents of rats given the meat diet contained significantly less acetate ($P < 0.01$), less butyrate ($P < 0.001$) and more isovalerate ($P < 0.001$) than those of control-fed rats.

Molar proportions of the SCFA in faeces and caecal contents for rats on the three diets are also shown in Table 3.

Caecal weights were measured in rats given the bran and meat diets. There was a significant difference between the two groups (33·7 (SD 3·3) and 16·7 (SD 2·2) g/kg body-weight respectively; $P < 0.001$).

Biopsies of liver, jejunum, ileo-caecal region and colon were taken for histology and pathology. Tissues were fixed in buffered formalin (100 ml/l), pH 7·0. Subsequent sectioning and staining (Haematoxylin and Eosin) were performed in the Research Pathology Laboratory, Western General Hospital.

There were no differences in histology of liver, jejunum, ileo-caecal region and colon between either of the test-diet-fed rats and the control-fed rats.

DISCUSSION

Excreted SCFA, hydrogen and methane are indicators of bacterial metabolism in the rumen and large intestine. The production of these fermentation products varies with the dietary composition (Hungate, 1968; Stevens, 1977). The rat, a single-stomached omnivore, is a suitable animal to feed with diets varying in composition for the study of bacterial metabolism in the caecum. In the present study three diets were chosen, a control laboratory diet, an essentially high-cereal-fibre herbivorous diet and a carnivorous diet.

Hydrogen production is known to fluctuate during the day depending on times of food consumption and reaches its maximum 4 h after feeding (Gumbmann & Williams, 1971). In our study each rat was tested at exactly the same time of day each week to eliminate any variations which might be attributed to the time of food ingestion. The different diets did not significantly alter hydrogen production.

Methane was detected only in the expired gas from control-fed rats, both males and females, at 12 weeks of age and thereafter. Rodkey *et al.* (1972) showed no statistically-significant difference between male and female rats in this respect.

Methanogenic bacteria appear to colonize the caecum and require a fibrous residue (McLean Ross *et al.* 1981), possibly to assist in colonization and to provide a potential source of substrate (Czerkawski & Breckenridge, 1979). There may be a critical period during which time anaerobic methanogens colonize the gastrointestinal tract (Savage, 1977; 1978). However, methane excretion was undetectable from rats on both the high-fibre and low-fibre test diets. An increased fibre content, although providing substrate, may alter bacterial fermentation patterns, or changes in the caecal environment may prevent or delay colonization by methanogens. Similarly, a lack of fibrous residue may also prevent or delay bacterial colonization.

Branched chain fatty acids resulting from microbial degradation of protein are required by several gut micro-organisms. *Methanobacterium ruminantium* requires 2-methylbutyric acid and acetate for growth (Prins, 1977). In the ruminant the production of methane and propionate are inversely related (Prins, 1979). There appear to be no such relationships between SCFA and methane production in the rat caecum.

Significant differences in the concentration of faecal SCFA were observed in male and female weanling rats, but not in adult animals. This may reflect a difference in the colonic bacterial population between the sexes at weaning, which subsequently alters.

In the present study, changes in diet are associated with changes in the concentration and proportion of faecal SCFA although the total faecal SCFA were similar. Dietary influences on faecal SCFA have been shown in the pig (Cranwell, 1968; Sambrook, 1979), in rodents (McBee, 1970) and in the dog, pig and pony (Stevens, 1977). The rat caecum contained a greater variety and higher concentration than faeces of SCFA. Caecal contents of rats given the bran diet showed higher SCFA (mean 398 mmol/kg) although the faeces were similar to controls. Caecal SCFA in rats given the meat diet were significantly lower than in the control rats. The caecal:faecal SCFA values were low in the meat-fed rats for all SCFA, which reflects a low SCFA production rate with little substrate.

Caecal production and absorption rates of SCFA control the concentrations and molar ratios. In the rumen, absorption rates of SCFA increase with chain length (acetate < propionate < butyrate) but the amount transported in the blood decreases (Stevens & Stettler, 1966); these differences are attributable to mucosal metabolism. Absorption of SCFA has been shown in the rat (Remesy & Demigne, 1976) and an estimated 4–7% of total energy requirement is obtained from SCFA produced in the caecum (Yang *et al.* 1970). The molar

proportions in the faeces from all rats and caecal contents in rats given the meat diet were similar to those reported by Remesy & Demigne (1976). Caecal contents reflected differences to a greater extent than faeces. This may indicate different SCFA absorption or metabolism rates which were influenced by diet.

A higher fibre content also led to an increase in caecal weight, an observation also reported by Ryan *et al.* (1979) and Murray *et al.* (1980).

It can be concluded that methanogenic bacteria may take several weeks to colonize the rat caecum significantly and this may be influenced by diet. Hydrogen, methane and SCFA excretion appear to be unrelated in the rat caecum and may reflect different aspects of bacterial metabolism.

REFERENCES

Bauchop, T. (1977). In *Microbial Ecology of the Gut*, pp. 223–250 [R. T. J. Clarke and T. Bauchop, editors]. London and New York: Academic Press.

Bryant, M. P. & Wolin, M. J. (1975). In *Proceedings of the First Intersectional Congress of the International Association of the Microbiological Society*, vol. 2. [T. Hasegawa, editor]. Tokyo: Science Council of Japan.

Clapperton, J. L. & Czerkawski, J. W. (1969). *British Journal of Nutrition* 23, 813–826.

Cranwell, P. D. (1968). *Nutrition Abstracts and Reviews* 38, 721–730.

Czerkawski, J. W. (1969). *World Review of Nutrition and Diet* 11, 240–282.

Czerkawski, J. W. & Breckenridge, G. (1969). *British Journal of Nutrition* 23, 925–937.

Czerkawski, J. W. & Breckenridge, G. (1979). *British Journal of Nutrition* 42, 229–245.

Gumbmann, M. R. & Williams, S. W. (1971). *Proceedings of the Society of Experimental and Biological Medicine* 136, 1171–1175.

Henning, S. J. & Hird, F. R. J. (1972). *British Journal of Nutrition* 27, 57–64.

Hobson, P. N. (1972). *Proceedings of the Nutrition Society* 31, 135–139.

Hungate, R. E. (1968). In *Handbook of Physiology*, Section 6, *Alimentary Canal*, vol v, pp. 2725–2745 [C. F. Code, editor]. Washington DC: American Physiological Society.

Hungate, R. E. (1976). In *Fibre in Human Nutrition*, pp. 131–149. [G. A. Spiller and R. J. Amen, editors]. New York: Plenum Press.

Kidder, D. E. & Manners, M. J. (1978). *Digestion in the Pig*. Bristol: Scientechnica, Bristol.

McBee, R. H. (1970). *American Journal of Clinical Nutrition* 23, 1514–1518.

McBee, R. H. (1977). In *Microbial Ecology of the Gut*, pp. 185–222 [R. T. J. Clarke and T. Bauchop, editors]. London and New York: Academic Press.

McLean Ross, A. H., McKay, L. F., Busuttil, A., Anderson, D. M. W., Brydon, W. G. & Eastwood, M. A. (1981). *Proceedings of the Nutrition Society* 40, 73A.

Murray, D., Fleiszer, D., McArdle, A. H. & Brown, R. A. (1980). *Surgical Research* 29, 135–140.

Murray, R. M., Bryant, A. M. & Leng, R. A. (1978). *British Journal of Nutrition* 39, 337–345.

Parker, D. S. (1976). *British Journal of Nutrition* 36, 61–70.

Parker, D. S. & McMillan, R. T. (1976). *British Journal of Nutrition* 35, 365–371.

Prins, R. A. (1977). In *Microbial Ecology of the Gut*, pp. 73–183. [R. T. J. Clarke and T. Bauchop, editors]. London and New York: Academic Press.

Prins, R. A. (1979). *Antonie van Leeuwenhoek* 45, 339–345.

Remesy, C. & Demigne, C. (1976). *Annals de la Recherche Vétérinaire* 1, 39–55.

Rodkey, F. L., Collinson, H. A. & O'Neal, J. D. (1972). *Journal of Applied Physiology* 33, 256–260.

Ryan, G. P., Dudrick, W. J., Copeland, E. M. & Johnston, L. R. (1979). *Gastroenterology* 77, 658–663.

Sambrook, I. E. (1979). *British Journal of Nutrition* 42, 279–287.

Savage, D. C. (1977). In *Microbial Ecology of the Gut*, pp. 277–310 [R. T. J. Clarke and T. Bauchop, editors]. London and New York: Academic Press.

Savage, D. C. (1978). *American Journal of Clinical Nutrition* 31, S131–S135.

Spiller, G. A., Chernoff, M. C., Hill, R. A., Gates, J. E., Nassar, J. J. & Shipley, E. A. (1980). *American Journal of Clinical Nutrition* 33, 754–759.

Stadtman, T. C. (1967). *Annual Review of Microbiology* 21, 121–125.

Stevens, C. E. (1977). In *Dukes' Physiology of Domestic Animals*, 9th ed, pp. 216–232 [M. J. Swenson, editor]. Ithaca: Cornell University Press.

Stevens, C. E. (1978). *American Journal of Clinical Nutrition* 31, S161–S168.

Stevens, C. E. & Stettler, B. K. (1966). *American Journal of Physiology* 210, 365–372.

Tadesse, K., Smith, A., Brydon, W. G. & Eastwood, M. A. (1979). *Journal of Chromatography* 171, 416–418.

Wolfe, R. S. (1971). *Advances in Microbial Physiology* 6, 107–146.

Yang, M. G., Manoharan, K. & Michelsen, O. (1970). *Journal of Nutrition* 100, 545–550.