Effects of Caecectomy on the Digestibility of Food and Rate of Passage of Digesta in the Rat

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Abstract

Minimum transit time through the alimentary tract of young adult female rats fed a stock diet *ad libitum* was reduced from $6 \cdot 6 \pm 0 \cdot 4$ h for sham-operated rats to $5 \cdot 0 \pm 0 \cdot 3$ h for caecectomized rats, but there was no effect on transit time of digesta along the small intestine. Caecectomy decreased the apparent digestibility of crude protein, soluble carbohydrate, cellulose and hemicellulose. Digestibility of lipid was not affected. However, caecectomized rats did not increase their dry matter intakes to compensate for the reduced digestible energy intakes.

Introduction

Ambuhl *et al.* (1979) found that caecectomy in rats reduced the apparent digestibility of crude protein and total carbohydrate in a stock diet high in grains but the separate carbohydrate fractions were not studied. Yang *et al.* (1969) reported that caecectomy in rats resulted in a reduction in digestion of the acid-detergent fibre in a diet high in ground corn. In this paper, the effects of caecectomy on the digestibilities of soluble carbohydrate, hemicellulose and cellulose in rats fed a diet containing a high content of grain were studied.

Iwai *et al.* (1973) reported that mice with enlarged caeca, due to manipulation of the gut bacterial flora, had slower transit rates of small intestinal contents than normal mice. Consequently it was thought that caecectomy may affect transit rates of digesta through the small intestine of rats. In order to assess minimum times digesta spent in the caecum first appearance of non-absorbable marker in the faeces and small intestinal transit times were also determined in sham-operated and caecectomized animals.

A summary of these studies has been reported (Williams and Senior 1981).

Materials and Methods

Animals and Environment

Young adult nulliparous black-hooded rats of approximately 60 days of age were used. One group was subjected to caecectomy and the other to sham-operation as previously described (Ambuhl *et al.* 1979). All animals were housed individually in metabolism cages at a temperature of 21–24°C with illumination from 0600 to 1800 h.

Feeding, Weighing of Animals and Collection of Faeces

A stock diet (Fielders Pty Ltd, Tamworth, N.S.W.) in powdered form was fed *ad libitum* (Table 1). Distilled water was always freely available. In digestibility trials faeces were collected for 5 days. The animals were weighed 2 days before and at the end of faecal collection. Faeces were collected

at 0900 h each day, dried at 97°C for 24 h and bulked for each rat. The total was dried for a further 24 h before weighing.

Chemical Analysis

Food and faecal lipid contents were determined by measuring the weight loss of ground samples after extraction with light petroleum (b.p. 40–70°C) for 24 h in a Soxhlet apparatus. The Kjeldahl technique using selenium as the catalyst was used for nitrogen determination. Dried, ground samples were oxidized in a muffle furnace for 1 h at 400°C followed by 18 h at 600°C in order to measure ash contents.

Hemicellulose, cellulose and lignin in food and faeces were determined by the measurement of neutral-detergent fibre, acid-detergent fibre and lignin (Van Soest 1963; Van Soest and Wine 1967). In order to reduce frothing during refluxing, and to facilitate filtering when determining neutral-detergent fibre, preliminary incubation with enzymes was used. Starch in food was hydrolysed with α-amylase (EC 3.2.1.1) (Calbiochem, La Jolla, California, U.S.A.) as recommended by McQueen and Nicholson (1979). This was of no advantage with faeces, but a reduction in frothing was obtained by incubation for 16 h at 37°C with 30 ml of trypsin (EC 3.4.4.4) (Hopkin and Williams, Chadwell Heath, England) 1 : 300 in phosphate buffer, pH 7, with 1 ml toluene.

The amount of nitrogen in neutral- and acid-detergent fibre was determined and a correction on the basis of $N \times 6 \cdot 25$ was made for residual protein.

Table 1. Constituents and chemical analysis of the stock diet

Constituent	Amount (g/kg)	Component by analysis	Amount (%)
Linseed meal	44	Ash	6·0
Maize grain	150	Lipid	3·8
Sorghum grain	160	Crude protein	21·7
Wheat grain	200	Soluble carbohydrate	46·6
Pollard	250	Hemicellulose	13·0
Bran	20	Cellulose	5·9
Lucerne meal	40	Lignin	3·0
Fish meal	50		
Meat meal	80		
Sodium chloride	3		
Vitamin supplement	3[A]		

[A] The vitamin supplement added the following: vitamin A, 1813 i.u./kg; vitamin D, 454 i.u./kg; vitamin E, 5 i.u./kg. The gross energy content = 18·7 kJ/g.

Statistical Analysis

Digestibility data were analysed by analysis of variance with repeated measures (Health Sciences Computing Facility, University of Los Angeles, California, U.S.A.) and Duncan's multiple-range test. Other means were compared by Student's *t*-test. All means are presented with their standard errors.

Food Intake and Digestion of a Stock Diet Fed ad libitum

Soluble carbohydrate digestibility was obtained by difference after accounting for lipid, crude protein, lignin and the other carbohydrate fractions in food and faeces. Determinations were made on each of the rats at 33, 70, 77, 84, 91 and 135 days after surgery. It was thus possible to determine whether anatomical adaptation of the colon subsequent to caecectomy affected food digestibility. Six sham-operated and six caecectomized rats were used.

First Appearance of Non-absorbable Marker in Faeces

Thirty-five days after surgery, the food fed contained $0 \cdot 35\%$ (w/w) Cr_2O_3. It was offered at 0900 h after a fast from 1700 h the previous day; all rats were observed to eat immediately. Faeces

were collected each hour, dried and ashed in a muffle furnace. The presence of marker was determined by visual inspection after breaking open the ashed pellets. The time of first appearance of marker was recorded as that at which a fully impregnated pellet had been excreted. Ten sham-operated and 12 caecectomized animals were studied.

Rate of Passage of Marker Along the Small Intestine

For the 2 days prior to administration of marker, the rats were allowed access to food from 0900 h to 1700 h only. On the morning of the third day food containing $2 \cdot 2\%$ (w/w) Cr_2O_3 was offered to each rat at a different time and the time a rat commenced eating was noted. Forty-five min, and other times up to 120 min, after an animal had eaten marker, a lethal intraperitoneal dose of sodium pentobarbitone was administered. The abdominal cavity was then opened, the leading edge of the Cr_2O_3 was located by visual inspection of the small intestine and a ligature was used to mark this point.

The small intestine was then removed, laid straight along a metre rule, and the total length, and distance from pylorus to ligature were recorded. The percentage of small intestine traversed by the marker was then calculated.

Gross Anatomy of the Colon

The digesta free weight and length of the colon were obtained immediately after killing for all 12 rats used in the digestibility study.

Results

Body Weight

All animals grew satisfactorily during the experiment. Caecectomized rats used for the digestibility study weighed $191 \cdot 4 \pm 2 \cdot 8$ g just before and $255 \cdot 8 \pm 7 \cdot 6$ g 135 days after surgery. Sham-operated rats weighed $192 \cdot 7 \pm 3 \cdot 3$ g before, and $267 \cdot 0 \pm 6 \cdot 6$ g 135 days after surgery.

Table 2. Digestibility of food components

n.s., not signficant

Food component	Mean digestibility coefficient $\pm$ s.e. (%)		P
	Sham-operated	Caecectomized	
Organic matter	$76 \cdot 7 \pm 0 \cdot 9$	$72 \cdot 0 \pm 1 \cdot 3$	$< 0 \cdot 0001$
Protein	$80 \cdot 1 \pm 1 \cdot 2$	$76 \cdot 7 \pm 1 \cdot 0$	$< 0 \cdot 0001$
Lipid	$89 \cdot 3 \pm 2 \cdot 5$	$89 \cdot 1 \pm 2 \cdot 7$	n.s.
Total carbohydrate	$74 \cdot 8 \pm 1 \cdot 1$	$69 \cdot 5 \pm 1 \cdot 9$	$< 0 \cdot 0001$
Soluble carbohydrate	$93 \cdot 2 \pm 0 \cdot 3$	$88 \cdot 4 \pm 0 \cdot 5$	$< 0 \cdot 0001$
Hemicellulose	$45 \cdot 2 \pm 1 \cdot 8$	$36 \cdot 2 \pm 1 \cdot 5$	$< 0 \cdot 01$
Cellulose	$18 \cdot 5 \pm 1 \cdot 6$	$10 \cdot 2 \pm 1 \cdot 2$	$< 0 \cdot 01$

Food Intake and Digestibility

The amount of food consumed per day was similar in both groups of rats whether expressed as per 100 g body weight or per 100 g body weight$^{0.75}$; the latter value was $24 \cdot 6 \pm 0 \cdot 4$ g for sham-operated and $24 \cdot 8 \pm 0 \cdot 6$ g for caecectomized animals. Analysis of variance showed that the digestibility coefficients of all food components other than lipid were greater for sham-operated than for caecectomized rats (Table 2). There were significant period differences for hemicellulose and soluble carbohydrate digestibility but they were random, indicating that this was not due to any anatomical adaptation of the colon.

First Appearance of Marker in Faeces

Chromic oxide appeared in the faeces of sham-operated animals $6 \cdot 6 \pm 0 \cdot 4$ h after eating. This time was reduced to $5 \cdot 0 \pm 0 \cdot 3$ h in caecectomized rats. The difference was significant ($P < 0 \cdot 01$).

Passage of Marker Along the Small Intestine

The percentage of the length of the small intestine traversed by chromic oxide 45 min after the commencement of eating was $82 \cdot 9 \pm 1 \cdot 3 \%$ in the sham-operated and $83 \cdot 9 \pm 1 \cdot 7 \%$ in the caecectomized rats. The difference was not significant.

Table 3. **Rate of passage of digesta along the small intestine of sham-operated and caecectomized rats**

	Sham-operated			Caecectomized	
Rat No.	Time after ingestion (min)	Length of small intestine travelled (%)	Rat No.	Time after ingestion (min)	Length of small intestine travelled (%)
734	55	91·3	741	90	92·7
736	65	92·5	743	105	100·0
738	75	89·5	745	105	100·0
739	105	100·0	746	105	100·0
742	105	100·0	747	105	97·4
744	105	100·0	749	120	100
748	105	100·0			
750	120	100·0			

Table 3 shows the results for longer times after eating. It is clear that it took about 105 min for marker to traverse the whole of the small intestine and enter the large intestine in both caecectomized and sham-operated rats. Digesta flow was approximately five times as fast in the first 80% of the small intestine as that in the final 20%.

Gross Anatomy of the Colon

The length of colon per 100 g body weight was $7 \cdot 1 \pm 0 \cdot 4$ cm in sham-operated and $8 \cdot 6 \pm 0 \cdot 5$ cm in the caecectomized rats 141–144 days after surgery. The difference was significant ($P < 0 \cdot 05$). The fresh weight of the colon per centimetre was $97 \cdot 0 \pm 5 \cdot 34$ mg in the sham-operated, and $108 \cdot 5 \pm 6 \cdot 4$ mg in the caecectomized animals. The difference was not significant and did not become significant when related to body weight.

Discussion

The determination of cell wall constituents by the neutral-detergent and acid-detergent fibre techniques has been criticized. Morrison (1980) analysed the carbohydrate composition of acid-detergent fibre from a range of plant materials and found it contained variable amounts of sugar units derived from hemicellulose. Thus the cellulose content was overestimated in fruit and root vegetables by 3–8%. It is

not considered that any errors of this magnitude would have seriously affected the calculated digestion coefficients in this present study.

Van Soest (1961) showed that proteins heated to 80–100°C were relatively insoluble in lauryl sulfate at pH 7·4, the detergent used in the estimation of neutral-detergent fibre. In this experiment both food and faeces were dried at 97°C and therefore corrections were made for the protein (N × 6·25) present in neutral- and acid-detergent fibre. The general trend in the data for the digestibility coefficients of the carbohydrate fractions was little affected by the correction.

The decreased apparent digestibilities of organic matter, crude protein and total carbohydrate due to caecectomy confirm the results of Ambuhl et al. (1979), and that of cellulose, the results of Yang et al. (1979). Decreased digestibility of the cell wall polysaccharides was expected as they are not subjected to endogenous enzyme digestion and absorption in the small intestine but they are fermented in the caecum. However, it was found that a significant fraction of soluble dietary carbohydrate was also fermented in the caecum of the sham-operated rats as shown by the decreased digestibility coefficient after caecectomy. Keys et al. (1969) showed that pigs and rats digested cellulose and hemicellulose in alfalfa to an appreciable extent but pigs digested these constituents in brome and orchard grasses better than rats. Lloyd et al. (1958) caecectomized pigs and fed combinations of high and low protein and fibre in four diets. Crude fibre digestibility was hardly affected when the diets were high (17%) in protein, but it was substantially decreased when the diets were low (8·7%) in protein. Gargallo and Zimmerman (1981) also caecectomized pigs and fed them diets containing 2, 10 and 18% cellulose as Solka-floc (powdered wood cellulose; Brown Co., New York, U.S.A.). They did not show any effect of caecectomy on cellulose digestion, probably because dietary protein content was sufficiently high (13·8%) even in the highest fibre diet.

Most of the digestion of cellulose in rats apparently takes place in the caecum, which is not unreasonable as this organ contains a high proportion of the volume of the large intestine in which fermentation takes place (Lange and Staaland 1970). However, it was surprising that caecectomy did not have a greater effect on hemicellulose digestibility (Table 2).

Yang et al. (1970) fed rats on a laboratory grain diet and estimated by an isotope-dilution technique that the caecum provided as volatile fatty acid, the end-product of carbohydrate fermentation, about 4·7% of the dietary energy or 9·4% of the energy requirement for maintenance. Farrell and Johnson (1970) carried out a similar experiment in pigs fed diets containing 8 or 26% cellulose and concluded that only 2·7 and 1·9% of the apparent digestible energy of the 26 and 8% cellulose diets respectively were accounted for by volatile fatty acid production in the caecum, which is proportionately less than occurred in the rat (Yang et al. 1970). In the pig it is possible that most fermentation occurs in the colon as in this species the caecum is only a small proportion of the large intestine (Colin 1871, cited by Hill 1970).

Ambuhl et al. (1979) showed that in the female albino rat derived from the Sprague-Dawley strain, caecectomy resulted in increased food intake to compensate for the lower digestibility of energy. It was therefore surprising that the black-hooded strain, which showed a similar fall in digestibility, did not compensate to the same extent. This suggests that the sensitivity of the hypothalamic centres controlling food intake is different in the two strains of rat.

Caecectomy caused the colon to increase significantly in length, but although the mean weight per unit length was greater after caecectomy, this effect was not significant. Ambuhl *et al.* (1979) showed the same, but more marked, effects of caecectomy in Sprague-Dawley rats.

Ambuhl *et al.* (1979) showed that the rate of decline in the concentration of the non-absorbable marker, ^{51}CrEDTA, in the faeces of caecectomized rats after a pulse oral dose, was faster than in sham-operated rats, indicating a faster rate of passage of digesta through the alimentary tract after caecectomy. The present data showed this effect in a different way: the time of first appearance in faeces of chromic oxide eaten with the food was about 5·0 compared to 6·6 h, or 32% faster, after caecectomy. An approximate measure of the minimum delay time of digesta in the caecum of this black-hooded strain is therefore 1·6 h.

The transit time of digesta along the small intestine was not influenced by caecectomy. Therefore the lower digestibility of the food caused by caecectomy could not have been due to any decreased time for digestion and absorption of nutrients in the small intestine; it appears to have been entirely due to the absence of caecal fermentation.

In the rat the relatively slow rate of transit through the ileum compared to the more proximal segments of the small intestine has been known for a long time (e.g. Reynell and Spray 1956). This is also true for other species such as the sheep (Grovum and Williams 1973). Possibly the most surprising result in transit times was the minimum period digesta apparently stayed in the colon. With an approximate 2-h interval to reach the caecum, 1·6 h in the caecum, an overall transit time of 6·6 h would mean that digesta resides for a minimum of 3 h in the colon. This allows substantial time for electrolyte and water absorption, major functions of this part of the gut.

In summary, the work reported in this paper showed that caecectomy in the rat does not affect the rate of movement of digesta in the proximal alimentary tract but digesta moves faster through the large intestine; fermentation time is decreased by a minimum of 1·6 h after caecectomy. All the carbohydrate fractions of a laboratory stock diet, soluble carbohydrate, hemicellulose or cellulose, and its crude protein content, are decreased in digestibility after caecectomy indicating that the caecum is a major site of fermentation of food residues in the rat.

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