

Effect of changing transit time on colonic microbial metabolism in man

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SUMMARY An investigation was made of the effect of changing mean transit time (MTT) by administration of drugs which affect colonic motility on faecal microbial mass in man. Senokot was used to accelerate and codeine and/or loperamide to prolong transit in subjects maintained on a constant high fibre diet. Doses of Senokot or codeine/loperamide were adjusted to halve or double transit time measured during a three week control period on diet alone. Stools were collected throughout and analysed for bacterial mass by a gravimetric procedure. Transit was measured by a continuous marker method. Senokot decreased mean transit time from 63.9 to 25.0 hours (n=6), with increased stool weight from 148 to 285 g/day. Bacterial mass increased in all subjects from a mean of 16.5 to 20.3 g/day (dry weight) ($p < 0.025$). Codeine/loperamide increased mean transit time from 47.1 to 87.6 hours (n=5), with decreased stool weight from 182 to 119 g/day. Bacterial mass decreased in all but one subject from a mean of 18.9 to 16.1 g/day (NS). There was a significant correlation between transit time and bacterial mass in all three periods ($r = 0.77$, $p < 0.001$). Changes in transit time are shown to alter microbial growth in the human colon and result in altered stool output, on a constant diet. Factors which affect transit may be as important as diet in determining large bowel function and hence susceptibility to disease.

For subjects living on western diets, the main identifiable component of human colonic contents and faeces is the microflora. The human colon has been shown to contain roughly 230 g of bacteria,¹ and, considering the metabolic activity, interactions and production and utilisation of nutrients that this represents, the importance of the colonic microflora as a specialised metabolic compartment with a wide variety of functions has only recently been realised.

Faeces, the final product of gastrointestinal activity, have been shown to comprise 55% bacteria on a dry weight basis for those consuming a western diet,² and as bacteria are about 80% water,³ this represents roughly 70% of wet stool weight. The long held assumption that the main component of stools is undigested dietary residues must therefore now be reconsidered.

The large proportion of bacteria in stools indicates that those factors which influence bacterial growth will have a dominating effect on total stool output. Indeed, the provision of carbohydrate, in the form of fermentable dietary fibre, results in increased bacterial growth, a large faecal bacterial mass and hence a larger stool bulk.⁴

Provision of substrate is not the only factor which influences bacterial cell growth, however. In studies where bacteria have been grown with a constant substrate supply, in *in vitro* continuous culture or in certain animal systems, such as the rumen, bacterial cell growth has also been found to depend on the turnover time, or dilution rate, of the system.⁵⁻⁸ In studies of faecal microbial mass in human subjects, we have observed that both faecal output and faecal microbial mass vary considerably, in spite of subjects being on identical dietary intakes.² This variation has been found to be related to transit time through the gut, and, consistent with the *in vitro* and ruminant studies, more bacteria were excreted the faster the turnover rate.⁹ It therefore seems possible that transit

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time through the colon has a marked influence on stool bulk in man independent of dietary intake. To test this hypothesis, we have altered the transit time of seven healthy volunteers by pharmacological means, while maintaining a constant dietary intake, and have measured the changes in faecal bacterial mass.

Methods

SUBJECTS

Seven healthy volunteers, four men and three women, aged 21–55 years, took part in the study. Six lived throughout the period of the study in the metabolic unit of the Dunn Clinical Nutrition Centre, Cambridge and carried out their normal work in or near Cambridge. The other subject lived at home, but received all his food daily from the metabolic kitchen and ate some of his meals there.

PROTOCOL

The study was divided into three three week periods, as shown in Figure 1. For the whole of this time the subjects ate a controlled diet, collected their faeces and took radio-opaque pellets with each meal for the measurement of mean transit time and as balance markers. During the first three week period the diet only was taken, then in the subsequent periods, either Senokot (to speed up transit) or codeine/loperamide (to slow down transit) were given in random order. Four subjects completed all three study periods while two managed control + Senokot and one control + codeine/loperamide. Faecal collections were continued for one week after the end of the study in order to recover all markers taken.

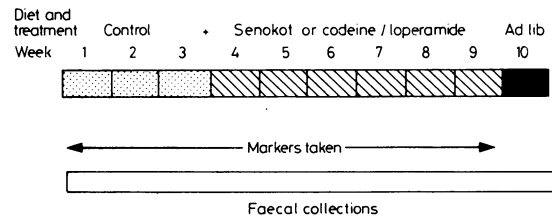


Fig. 1 Protocol of the study. Diet only was given for the first three weeks. Senokot and/or codeine/loperamide were then given in random order for subsequent three week periods.

DIET

The diet was constant throughout the study, and consisted of three one day menus of similar composition fed in rotation (Table 1). The composition of this diet, determined from food tables,¹⁰ was: energy 9.7 MJ, protein 91.5 g, fat 82.0 g and carbohydrate 324 g. The diet was designed to contain twice the normal daily intake of dietary fibre consumed in the United Kingdom, most recently estimated as 14.7 g/day.¹¹ This high level of fibre was required to provide a sufficiently large faecal microbial mass and sufficiently fast transit time on the diet alone such that these could be altered during the codeine/loperamide treatment without distress to the participants. The energy requirements of each subject were estimated using FAO/WHO recommendations based on ideal weight for height.¹² To meet these energy needs the subjects' energy intake was adjusted by increasing or decreasing the intake of certain foods incrementally. One increment (0.75 MJ) contained 20 g milk, 20 g sugar, 3 g butter, 1 g mayonnaise, 10 g meat or fish of the day, and 15 g ice cream (day 1), 20 g custard (day 2) and 25 g rice pudding (day 3).

Table 1 Control diet

<i>Daily</i>		
Wcctabix – 35 g		Pear (no skin or core) – 100 g
Wholemeal Bread – 100 g		Banana – 100 g
*Milk – 150 g		Lettuce – 15 g
*Mayonnaise – 18 g		Beetroot – 30 g
*Sugar – 40 g		Cucumber (no skin) – 20 g
Jam/marmalade (jelly) – 15 g		Gravy – 60 g
Cake (date, apple or banana) – 60 g		
Apple (no skin or core) – 100 g		
<i>Day 1</i>	<i>Day 2</i>	<i>Day 3</i>
*Turkey (breast) – 60 g	*Salmon (tinned) – 75 g	*Pork (lean) – 60 g
Coleslaw – 140 g	Potato Salad – 140 g	Baked Beans (tinned) – 50 g
*Mayonnaise – 18 g	*Mayonnaise – 18 g	*Mayonnaise – 16 g
Apricots (tinned, no syrup) – 120 g	Peaches (tinned, no syrup) – 120 g	Pineapple (tinned, no syrup) – 120 g
Lentil soup – 200 g	Leek & potato soup – 200 g	Pea & ham soup – 200 g
*Pork (lean) – 80 g	*Chicken (breast) – 90 g	*Beef (lean) – 80 g
Broccoli (frozen) – 70 g	Baked beans (tinned) – 80 g	Runner Beans (frozen) – 50 g
Carrots (tinned) – 70 g	Sprouts (frozen) – 100 g	Carrots (tinned) – 50 g
Potatoes (fresh) – 150 g	Potatoes (fresh) – 100 g	Potatoes (fresh) – 150 g
*Ice cream (dairy) – 50 g	*Custard (tinned) – 100 g	*Rice pudding (tinned) – 100 g

*Foods adjusted in increment.

Adjustments to energy intake were made only during the first week of the study. The increment was designed so as to contain only minimal quantities of non-starch polysaccharide. The energy requirements of the subjects varied from 8.1 MJ (-2 increments) to 15.7 MJ (+8 increments). For each level of energy intake duplicate portions of each of the components of the diet were collected at the beginning and end of the study. These were homogenised, freeze dried, and then stored before analysis.

ADMINISTRATION OF DRUGS

The diet was designed to achieve a transit time of about 48 hours. The aim of drug treatment was to approximately double or halve this time.

To speed up transit, Senokot tablets (Reckitt & Colman) each containing the equivalent of 7.5 mg sennoside B were used. Initially two tablets were given with breakfast, the dose being increased by one tablet daily until the desired effect was achieved.

Transit was slowed down using codeine phosphate (15 mg tablets) in combination with loperamide (2 mg tablets) (Janssen Pharmaceuticals Ltd). An initial dose of 30 mg codeine phosphate was given (15 mg morning and night) and the dose was increased to 120 mg daily if necessary. If this did not produce an appropriate slowing of transit, the dose was reduced to 60 mg per day and loperamide added until transit was slowed. All adjustments were made during the first week of each three week period.

The final doses of drugs were: Senokot; three subjects three tablets daily and three subjects four tablets daily; codeine/loperamide; one subject 30 mg codeine, two subjects 60 mg codeine, one subject 60 mg codeine + 4 mg loperamide, one subject 60 mg codeine + 8 mg loperamide.

FAECAL COLLECTIONS

Throughout the diet period and for one week after, subjects collected all stools. Each stool was collected separately into a plastic bag suspended over a toilet and was immediately frozen. Each stool was then weighed and radiographed for radio-opaque markers. The stools were pooled into seven day collections, weighed and freeze dried in an Edwards EF6 shelf freeze dryer for one week. On removal, the pooled collections were reweighed, allowing calculation of faecal solids (dry weight) and % dry weight. The stools were then crushed with a rolling pin, mixed and aliquots taken for measurement of faecal carbohydrate and microbial solids.

FAECAL MARKERS AND TRANSIT TIME

Throughout the study subjects took 30 radio-opaque pellets per day (10 per meal) as a non-absorbable

marker.¹³ Two shapes of marker were used, small rods and small circles, both made from radio-opaque tubing (Portex Ltd, Hythe, Kent). The type of marker was changed at the end of each three week period, so that the total recovery of marker during each diet period could be estimated. The number of markers present in each stool was counted after radiographing. Stool weight and faecal excretion of microbial solids and carbohydrate were corrected for marker output by multiplication of the mean daily output for the third week of each period by the ratio of marker output to intake for that week.

Transit measurements were made by the continuous marker technique of Cummings *et al.*¹⁴ The same radio-opaque pellets which were used as balance markers were used for the transit calculations. Measurement of marker excretion in each stool allows a day by day continuous record of mean transit time. These daily results were used to adjust the dosage of drugs until satisfactory slowing or speeding up of transit had occurred.

CHEMICAL AND MICROBIOLOGICAL METHODS

Dietary fibre was measured by the method of Englyst,¹⁵ which measures by gas liquid chromatography, the individual sugar components which make up non-starch polysaccharides (NSP). In the Englyst procedure, three samples of food are analysed by different but complementary procedures:

Sample A

After gelatinisation and incubation with hog pancreatic amylase and pullulanase to remove starch, the residue is solubilised in 12M H₂SO₄, hydrolysed with M H₂SO₄, and the individual neutral sugars measured by gas liquid chromatography. Uronic acids are measured separately by the method of Wardi *et al.*¹⁶ Using these procedures a measure of total NSP is obtained as well as any starch resistant to hydrolysis by α -amylase.

Sample B

After an identical starch removal procedure as in A above, the sample is hydrolysed with M H₂SO₄ and individual sugars are determined as in procedure A. This estimates non-cellulosic polysaccharides (NCP). The value for cellulose is obtained by subtracting the glucose in NCP from glucose measured in sample A (NSP).

Sample C

After starch removal the material is extracted with buffer at pH 7, the residue is solubilised with 12 M H₂SO₄, hydrolysed with M H₂SO₄ and individual sugars measured as in sample A. This gives a value for NCP insoluble in water at pH 7. A standard

sample of rye biscuit was run with each batch of dietary samples.

TOTAL CARBOHYDRATE IN FAECES

Total faecal carbohydrate content and the composition of individual monosaccharides was measured by the method of Wiggins.¹⁷ In this method duplicate aliquots of freeze dried faeces are heated in a boiling water bath with 0.5M H₂SO₄. The sugars released are estimated by gas liquid chromatography as alditol acetates. The residue is then treated with 72% H₂SO₄ for 48 h at 4°C and the hexose released gives a value for cellulose. Digestibility of individual monosaccharides were calculated as:

$$\frac{\text{intake} - \text{excretion}}{\text{intake}} \times 100$$

FAECAL FRACTIONATION

The bacterial fraction of stool was estimated on unground dried stools as previously described.² The fractionation is achieved by repeated mixing in a Colworth Stomacher 80, of 0.5 g dried stool combined with formylsaline (0.9% w/v NaCl and 1% v/v formalin) and Sodium Lauryl Sulphate (0.1%) followed by filtering through graded nylon meshes. This procedure gives a fraction of coarse particles mainly of plant cell wall material, a fraction of fine particles which are also from the plant cell wall and the suspension of bacteria in a large volume of washings. Each fraction is centrifuged at 30 000 g for 30 minutes on a MSE HiSpin 21 centrifuge. After discarding the supernatant, pellets are dried to constant weight.

The amount of water soluble material in faeces was determined using a similar procedure, but without detergent. The material was agitated with formylsaline for ten minutes and then centrifuged at 30 000 g for 30 minutes. The weight of the pellet which resulted after drying was subtracted from total solids to give water soluble material in the stool. The fractionation procedure was carried out on duplicate samples of freeze dried stool.

The results in the paper are from the final seven

days of each study period and Student's *t* test has been used to indicate the significance of differences between dietary periods.

Results

DIETARY FIBRE INTAKES

The diet was designed so that all the subjects, whatever their energy intakes, should have similar intakes of dietary fibre. Three diets which covered the range of energy intakes (-2 increments, +4 and +8, equivalent to 8.1 MJ, 12.7 MJ, and 15.7 MJ), were chosen for analysis of NSP. Table 2 shows that NSP intake was 29.4 ± 1.4 g/day, confirming that the diet provided twice the normal UK intake of dietary fibre. There was little variation amongst the three diets or amongst the three daily menus. Of the total NSP, 44% were water soluble polysaccharides.

MEAN TRANSIT TIME

Of the 3780 markers taken by the subjects during the study 3762 (99.5 ± 0.4%) were recovered, allowing accurate estimates of mean transit time. On the control diet, despite all the subjects being on identical NSP intakes, there was a considerable range of mean transit time, average 61 ± 30 h (range 35–125 h). The men had faster mean transit time (45 h) than the women (81 h) although this difference was not statistically significant. Table 3 shows the changes in transit after administration of Senokot or codeine/loperamide. A change in mean transit time in the desired direction was achieved in all subjects. On average, the changes in transit time which occurred with the two drug treatments were as intended: approximately half the transit time with Senokot (64 hours to 35 hours) and double the transit with codeine/loperamide (47 hours to 88 hours).

The mean transit time method allows a day to day measure of transit to be made once a steady state has been achieved. Figure 2 shows the daily values for two subjects during the three different experimental periods. Considerable variation occurs from day to

Table 2 Analysis of the diet for non-starch polysaccharides (g/day)

	Day 1	Day 2	Day 3	Mean
NCP	19.6 ± 0.6	21.4 ± 2.2	20.9 ± 1.2	20.6 ± 0.9
Hexose	5.3 ± 0.3	6.1 ± 0.6	5.8 ± 0.4	5.7 ± 0.4
Pentose	10.5 ± 0.2	11.4 ± 0.6	11.2 ± 0.6	11.1 ± 0.5
Uronic Acid	3.9 ± 0.2	3.9 ± 1.1	3.8 ± 0.7	3.9 ± 0.1
Cellulose	8.5 ± 0.5	9.4 ± 0.8	8.3 ± 0.2	8.7 ± 0.6
Total NSP	28.2 ± 0.2	30.8 ± 2.3	29.2 ± 1.1	29.4 ± 1.4

NCP=non-cellulosic polysaccharides=hexose (other than from cellulose) + pentose + uronic acids; NSP=non-starch polysaccharides=NCP + cellulose.

The Table shows the mean values (±SD) for three diets analysed which covered the range of energy intakes in the study.

Table 3 Mean transit time (h) for control, Senokot and codeine/loperamide periods (average value for the last 7 days of each study period)

Subject	Sex	Control	Senokot	Codeine/ loperamide
1	M	35	23	54
2	F	55	30	132
3	M	52	24	115
4	F	64	46	-
5	M	52	34	85
6	F	125	53	-
7	M	41	-	52
Mean (1)		63.9	35.0†	
SEM		12.8	5.0	
Mean (2)		47.1		87.6*
SEM		3.8		16.1

Mean (1) - Mean for subjects who took Senokot; mean (2) - Mean for subjects who took codeine/loperamide. * $p < 0.05$; † $p < 0.025$.

Table 4 Mean daily faecal weight (marker corrected) for control, Senokot and codeine/loperamide periods

Subject	Sex	Control	Senokot	Codeine/ loperamide
1	M	268	460	156
2	F	137	275	91
3	M	148	369	109
4	F	98	185	-
5	M	141	244	97
6	F	93	178	-
7	M	217	-	144
Mean (1)		147.5	285.3†	
SEM		26.0	45.1	
Mean (2)		182.3		119.3
SEM		26.1		13.0

Mean (1) - Mean for subjects who took Senokot; mean (2) - Mean for subjects who took codeine/loperamide * $p < 0.025$; † $p < 0.005$.

day and week to week in mean transit time despite diet and drugs being kept constant after week 1.

FAECAL COMPOSITION

The changes in mean transit time resulted in significant alterations in mean daily stool output (Table 4). On the control diet, stool output varied from 85 to 268 g/day with the men having significantly higher outputs (194 ± 61 g/day) than the women (109 ± 24 g/day) ($p < 0.05$). Stool weight increased from 148 g/day to 285 g/day (93%) with Senokot and fell from 182 g/day to 119 g/day (45%) with codeine/loperamide. Faecal weight and mean transit time were closely related ($r = 0.94$, $p < 0.001$) ($n = 18$). The % dry weight of stools also varied with transit time for all three periods ($r = 0.86$, $p < 0.001$) ($n = 18$).

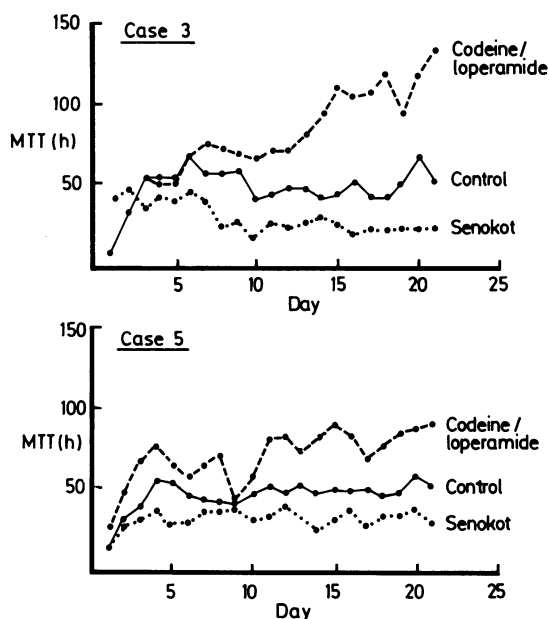


Fig. 2 Mean transit time (MTT) (h) measured by the continuous marker method in two individual subjects during the control, Senokot and codeine/loperamide periods.

●—● control ●····● Senokot ●- - -● codeine/
loperamide.

MICROBIAL SOLIDS

Microbial solids were the largest fraction of faecal solids ($45.8 + 3.9\%$) on the control diet. This proportion is not as great as in previous studies, where bacteria were found to represent 55% of faecal solids,² because the greater fibre content of the present diet meant that a greater proportion of faecal solids consisted of undegraded fibre.

Table 5 shows the changes in excretion of the components of bacterial solids with the Senokot and codeine/loperamide treatments. Faecal bacterial mass increased in every subject with the Senokot treatment, the mean change being from 16.5 g/day (dry weight) to 20.3 g/day ($p < 0.025$). With codeine/loperamide, faecal bacterial mass decreased in all but one subject, the mean change being 18.9 g/day to 16.1 g/day (NS). A close relationship was found between microbial mass and mean transit time over all 3 periods ($r = 0.77$, $p < 0.001$) (Fig. 3).

Table 5 also shows that the excretion of the water soluble fraction of stools increased with Senokot from 8.9 to 15.1 g/day ($p < 0.001$) and decreased with codeine/loperamide, from 9.2 to 7.3 g/day ($p < 0.05$).

FAECAL CARBOHYDRATE EXCRETION

The excretion of the individual sugar components making up the faecal carbohydrate are shown in Table 6. Total faecal carbohydrate excretion for the

Table 5 Daily excretion (g/day) of faecal solids (faecal dry weight), soluble material and bacteria for control, Senokot and codeine/loperamide periods

Control			Senokot			Codeine/loperamide			
Subject	Solids	Soluble	Bacteria	Solids	Soluble	Bacteria	Solids	Soluble	Bacteria
1	47.9	11.6	24.6	60.8	17.6	25.9	42.2	7.9	18.6
2	33.5	7.0	14.1	45.8	13.9	20.2	28.6	5.7	12.1
3	37.8	10.5	17.6	58.0	18.7	25.1	38.1	7.1	19.3
4	29.8	7.8	13.0	39.2	13.3	15.6	—	—	—
5	38.1	8.6	17.7	47.5	15.9	20.0	32.8	7.3	16.0
6	29.5	7.9	12.0	37.1	11.3	15.1	—	—	—
7	41.7	8.4	20.6	—	—	—	32.8	8.3	14.6
Mean (1)	36.1	8.9	16.5	48.1‡	15.1§	20.3‡			
SEM	2.8	0.7	1.9	3.9	1.1	1.9			
Mean (2)	39.8	9.2	18.9				34.9*	7.3*	16.1 ^{NS}
SEM	2.4	0.8	1.8				2.4	0.4	1.3

* $p < 0.05$; † $p < 0.025$; ‡ $p < 0.005$; § $p < 0.001$.

control period was about 7 g/day. Of this, the pentoses, arabinose and xylose, and the cellulose are exclusively fibre residue. The remaining sugars may also be derived from fibre, although some, particularly ribose and some of the glucose, are likely to be bacterial in origin. With Senokot, the excretion of total faecal carbohydrates increased from 6.5 g/day to 10.7 g/day, caused mainly by an increase in the excretion of cellulose, from 2.4 to 4.6 g/day ($p < 0.05$) and non-cellulosic glucose, from 0.6 to 2.0 g/day ($p < 0.05$). There was no significant effect of codeine/loperamide on faecal carbohydrates although both

non-cellulosic glucose and cellulose excretion fell in all subjects except one.

On the control diet, the digestibilities of the individual dietary carbohydrates were: arabinose 76%, xylose 76%, and cellulose 71%. With Senokot, cellulose digestibility fell significantly from $72.4 \pm 2.8\%$ to $47.7 \pm 10.6\%$ ($p < 0.05$). No significant changes in the digestibility of the other sugars were seen, nor were any significant differences observed in the codeine/loperamide period.

Discussion

It is a widely held view at present that diet is the principal factor controlling large bowel function. As a result, it has been suggested that diet may play an important part in determining susceptibility to colonic disease in different populations in the world.¹⁸ Whilst good experimental evidence exists to show that altering dietary intake, especially of dietary fibre, alters bowel function significantly,¹⁹⁻²² there may be other important determinants of bowel function which could similarly influence susceptibility to disease.

In studies of bowel function of healthy individuals, we have observed marked differences between subjects despite their being on identical or similar diets.²³⁻²⁵ A three-fold range in stool weight, mean transit time,²³ faecal ammonia concentration²⁴ and bile acid excretion²⁵ has been found in healthy young men consuming diets which were virtually identical, while these ranges are even wider when more heterogeneous groups are studied.^{25,26} Moreover, prolonged studies show that bowel function varies significantly from week to week in single individuals on constant diets.

For those eating a typical low fibre western diet, the principal component of human faecal and colonic

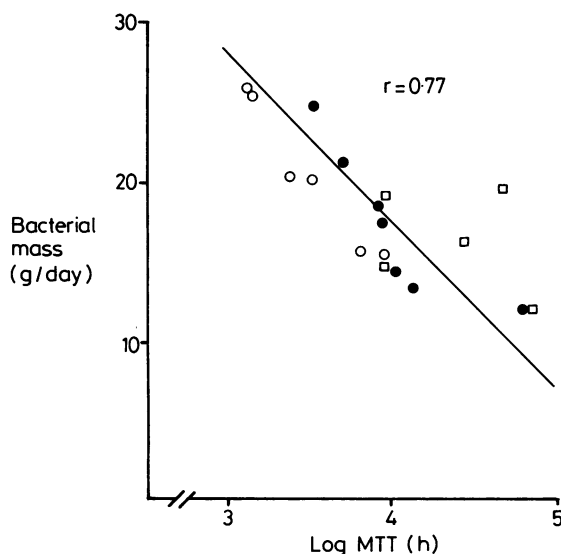


Fig. 3 Relationship between daily faecal excretion of bacteria (g dry weight/day) and the log mean transit time (MTT) (h). $r = 0.77$, $p < 0.001$. ● control ○ Senokot □ codeine/loperamide.

Table 6 Excretion of faecal carbohydrates (g/day)

	<i>n</i>	<i>Rha</i>	<i>Fuc</i>	<i>Rib</i>	<i>Ara</i>	<i>Xyl</i>	<i>Man</i>	<i>Gal</i>	<i>Glu</i>	<i>Cellulose</i>
Control (1)	6	0.2	0.1	0.1	1.2	1.4	0.1	0.4	0.6	2.4
SD		0.04	0.05	0.05	0.1	0.1	0	0.1	0.5	0.6
Senokot	6	0.2	0.1	0.1	1.3	1.7	0.1	0.6	2.0*	4.6*
SD		0.05	0.07	0.1	0.5	0.6	0	0.2	1.4	2.3
Control (2)	5	0.2	0.1	0.1	1.2	1.4	0.1	0.5	1.1	2.6
SD		0.08	0.04	0.04	0.1	0.2	0	0.1	0.8	0.5
Codeine/loperamide	5	0.2	0.1	0.1	1.3	1.4	0.2	0.4	0.6	2.2
SD		0	0.2	0.08	0.1	0.1	0.3	0.1	0.3	0.3

* $p < 0.05$; Rha=rhamnose; Rib=ribose; xyl=xylose; Gal=galactose; Fuc=fucose; Ara=arabinose; Man=mannose; Glu=glucose.

contents is the microflora.^{1,2} The control of microbial metabolism is therefore a key factor in explaining variations in colonic function. Because of the inaccessibility of the human colon, it is difficult to show changes in microbial metabolism in the gut itself, and it has been necessary to study the product of colonic metabolism, namely stools. By using a gravimetric procedure developed from animal work, it has been possible to investigate the effect of changes in diet on the size of the faecal bacterial population and we have shown that the colonic microflora responds to increases in fermentable carbohydrate supply by increasing cell numbers and mass. Numerous studies in the ruminant and *in vitro* have shown, however, that the efficiency of anaerobic microbial growth is dependent, not only on substrate availability, but also on the rate of passage of material through the fermentation system. With a faster turnover rate, the more efficient is microbial growth and a greater mass of bacteria is produced. This is thought to be because the maintenance requirement of the resident bacterial population is reduced, and more of the available energy supply can be used for bacterial cell growth.

The maintenance requirement of bacteria is always met in preference to growth and is that energy used for motility, replacement of lysed cells, maintenance of intracellular solute concentrations, active transport and turnover of intracellular constituents.⁶ With changing dilution rates and turnover times, maintenance requirements may alter for a variety of reasons, for example if the size of the resident population changes, if the resident bacteria are in a different phase of growth, or if the resident bacteria are subjected to stressful environmental conditions, such as high concentrations of NH_4Cl .⁶ Irrespective of the reason for a change in the maintenance energy requirement, the result of such a change is to alter the amount of energy available for new cell growth.

The present investigation set out to determine whether bacterial growth in the human colon is similarly influenced by changes in turnover time. We examined this possibility by changing the transit time

of healthy volunteers maintained on constant diets. Transit was altered using drugs which primarily affect gut motility and are not known to influence microbial activity directly. The drugs had to be effective, lack side effects, and be tolerated well by volunteers over extended periods. Senokot has been safely used for decades to treat constipation²⁷ whilst codeine phosphate is a well established drug commonly used to slow transit in diarrhoea. It was found, however, that speeding up transit was far easier than slowing it. The initial two tablets of Senokot were invariably not enough but once the dose was increased to three or four tablets per day a sustained rapid transit was achieved (Fig. 2). To slow transit time all but one subject (the only woman) required more than 30 mg/day of codeine phosphate. Two subjects slowed with 60 mg/day but the other two had to be given loperamide in addition. Loperamide is a new anti-diarrhoeal agent which slows transit by a direct opiate-like inhibitory effect on peristaltic activity in the gut wall.^{28,29} It does not influence fluid and electrolyte transport in unstimulated gut mucosa.³⁰

By altering the doses of these drugs appropriately, the desired changes in mean transit time were achieved, and significant alterations in colonic function were observed. Stool weight was increased with Senokot and reduced with codeine/loperamide. As in other studies where transit time and stool weight have been measured, an inverse relationship between the two was found.

The main purpose of the study was to investigate changes in microbial mass when transit was changed. With Senokot, the microbial fraction of stools was increased significantly, from 16.5 to 20.3 g/day, an increase of 25%. With codeine/loperamide, the microbial fraction fell from 18.9 to 16.1 g/day (20%), although this difference was not significant. These results suggest that, as predicted, altering transit time can result in a change in faecal microbial mass. Furthermore, a close relationship was found between transit time and microbial mass (Fig. 3), indicating that even with manipulations of transit using drugs,

the excretion of bacteria remains closely linked to the turnover time of the colon.

That these results reflect changing efficiency of bacterial cell growth depends on the supply of substrate being constant, which can be judged in part by examination of the intake and excretion of fermentable carbohydrate. With a constant diet throughout the study, dietary fibre and starch intakes were constant for each subject; from subject to subject, intakes of dietary fibre and starch also varied little, as foods containing dietary fibre were not included in the dietary increments, and the only incremented item which contained starch was the pudding on each day. The variation in starch intake from the lowest to highest energy intake was therefore only 9 g.

The faecal excretion of carbohydrate (Table 6), shows that the total excretion of carbohydrate increased with Senokot, through reduced digestibility of cellulose and NCP glucose. Arabinose and xylose, the main cell wall pentose sugars in plants, were unchanged. No change in excretion or digestibility was observed with codeine/loperamide, probably because digestibility was maximum on the control diet with mean transit time over 50 h. Several studies have indicated that, in man, mean transit time affects digestibility only if it is less than 50 h.^{23,31} The increased microbial mass seen with Senokot therefore occurred at the same time as reduced fermentation of NSP.

The difficulty is assessing the constancy of the substrate supply lies in determining whether the drugs used alter the amount of fermentable carbohydrate entering the large intestine. Non-starch polysaccharides are not degraded in the small intestine;³² hence the amount entering the large intestine is constant for control, Senokot and codeine/loperamide periods. Whether degradation of starch is affected by changes in transit time is not clear. In an investigation of starch malabsorption in healthy volunteers using an intubation technique, no relationship was found between the amount of starch escaping degradation and absorption in the small intestine and the time taken for the test meal to pass through the terminal ileum.³³ Studies on ileostomists, however, have indicated that the extent of starch malabsorption is affected by changing small intestinal transit time.^{34,35}

The basal diet in the present study contained 133 g starch (range for subjects 131 g–140 g). Recent studies suggest that 5–10% of dietary starch may escape absorption in the small intestine;^{33,36} such a proportion means that about 8–12 g of starch would enter the colon on the present diet. An effect of loperamide to halve this amount, as suggested by Chapman *et al.*,³⁴ could account for some, but not

all of the change in bacterial mass which occurred when transit was slowed. The relative importance of changes in substrate supply and altered efficiency of microbial cell growth remains to be clarified.

This study has shown that transit time has a major controlling influence over the colonic microflora and therefore over colonic function. Differences in bowel habit and microbial cell metabolism between individuals on similar diets are largely attributable to differences in mean transit time. Moreover the changes in bowel habit seen in these people due to mean transit time are of the same order or greater than those which can be induced by dietary manipulation. If diet is an important determinant of bowel function and disease so also must transit be, both acting through an effect on the microflora. The factors controlling transit itself, whether environmental or genetically determined, must therefore be important in affecting bowel function in both health and disease.

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