Alimentary tract and pancreas

Degradation of cellulose within the gastrointestinal tract in man

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SUMMARY ^{14}C -labelled cellulose was administered orally to 10 subjects without gastrointestinal disease and its absorption measured by faecal ^{14}C excretion and $^{14}\text{CO}_2$ in expired air. A mean of 57.2% of oral cellulose was excreted in the faeces and up to 14% (mean 7.5 ± 4.4 1SD) of faecal radioactivity was water soluble. Whole gut transit time did not correlate with the quantity of ^{14}C -cellulose excreted in the faeces. A significant quantity of $^{14}\text{CO}_2$ appeared in the expired air as early as 30 minutes after administration of the labelled cellulose. The cumulative excretion of $^{14}\text{CO}_2$ varied from 7.6–32.2% of the administered radioactivity but did not correlate with faecal ^{14}C excretion. The present data show that a significant quantity of oral cellulose is metabolised within the human gastrointestinal tract and appears in the expired air as $^{14}\text{CO}_2$.

In a recent review on dietary fibre the uncertainties in relation to digestion of the different types of fibre by the human gastrointestinal tract have been highlighted.¹ There is a striking variation in the apparent digestibility of different dietary fibres. Some such as pectin and hemicelluloses are virtually completely digested while much more uncertainty surrounds the digestibility of cellulose. Southgate and Durnin² reported that cellulose digestion in young men and women averaged approximately 20% and Prynne and Southgate,³ reported a mean digestion of 72% in a similar group. The presence of lignin included in the cellulose assay of the former study was thought to be a factor in explaining this discrepancy between the two studies. Other workers have shown similar variation.⁴

While great progress has been achieved in improving the reliability and specificity of measurements of dietary fibre⁵ these methods have been designed and applied almost exclusively to food analysis. In balance studies where faecal analysis of non-digested or partly digested fibre is required, the specificity of these methods is not proven. Whether the great variation in cellulose digestion is a true biological variation resulting from different gut microflora or is due to the physical form of the ingested cellulose or to unsuitable methodology is unclear.

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Using an isotopically labelled native cellulose preparation, we have performed balance studies on human subjects in an attempt to further define the extent of cellulose digestion and absorption within the human gastrointestinal tract.

Methods

SUBJECTS

Ten volunteer subjects, to whom the purpose and nature of the study was explained in detail, were studied after they had given informed consent. Six elderly subjects (age range 79–82 years) and four younger subjects (age range 39–50 years) were studied. All were consuming mixed normal diets and were without symptoms or history of gastrointestinal disease. None were taking any antibiotics or drugs known to influence gastrointestinal function.

EXPERIMENTAL PROTOCOL

Subjects having fasted overnight were administered a standard oral dose of cellulose made up as follows. Five μCi (185 kBq) of ^{14}C -cellulose (specific activity 0.15–0.2 $\mu\text{Ci}/\text{mg}$) was intimately mixed with 15 g of instant mashed potato (Dornay Foods Ltd). Hot water was then added to reconstitute the mashed potato and this was administered by spoon. The subjects were closely observed during administration to ensure complete consumption of the administered dose which was subsequently followed

by a cup of tea. The total quantity of cellulose taken was approximately 500 mg. Normal meals were allowed from then on. Twenty four radio-opaque pellets were administered simultaneously with the cellulose dose to assess transit time⁶ and completeness of the subsequent faecal collections.

All faecal samples collected individually were double wrapped in polyethylene bags and labelled with name, date, and time of collection. Faecal samples were stored at -20°C for subsequent analysis.

Samples of expired air were collected by blowing through a standardised volume of hyamine hydroxide containing phenolphthalein as indicator;⁷ this trapped 1 mmol of CO_2 . Single breath samples were collected at 15 minute intervals for the first four hours, and thereafter duplicate samples were taken as follows: 30 minute intervals for a further eight hours, two hourly for a further 12 hours, six hourly for a further 24 hours and, finally, 12 hourly for 120 hours, giving a total collection time of seven days.

¹⁴C-LABELLED CELLULOSE

This was supplied by ICN Chemicals and Radioisotopes (Laboratory Impex Ltd, UK). The source material was prepared from *Cana indica* leaves which were allowed to photosynthesise in an atmosphere of $^{14}\text{CO}_2$ for 24 hours. The leaves were then treated as follows. Pigments and soluble polysaccharides were removed with hot 80% alcohol. Pectin was removed by refluxing at pH2 with dilute hydrochloric acid, filtering while still hot and washing with hot water followed by ethanol. The residue was dried in a desiccator and lignin removed by stirring for several hours in acetic acid, sodium chlorite solution, followed by removal of the acid with water and then with ethanol. The residue was again dried and hemi-celluloses removed by stirring in a 10% sodium hydroxide solution containing 1% boric acid for 24 hours at 25°C under nitrogen. The cellulose was filtered and washed free of alkali with water followed by alcohol and then dried. This preparation as supplied is stated to be free of lignin and hemi-celluloses.

The commercially prepared material was examined microscopically after iodine staining and was seen to contain numerous starch granules. Further analysis using thin layer chromatography after amylase digestion showed that the starch component was isotopically labelled and therefore had to be removed before the start of the cellulose digestion studies. The following procedure was developed.

The cellulose is autoclaved at 15 psi, 115°C for 20 minutes for subsequent starch digestion. The

preparation is then washed with water and incubated with 1,4- α , 1,6- α amyloglucosidase EC 3.2.1.3 (from *Rhizopus* mold, Sigma Chemical Co Ltd) in phosphate buffer pH 4.5 for five hours. Copious dilute hydrochloric acid washing followed by washing with water was then performed to remove the residual enzyme and glucose product. All washings were assayed for C^{14} content and for microbiological sterility. This procedure removed approximately 30% of the original radioactivity and further microscopic examination after iodine staining revealed only trace quantities of starch granules. Semi-quantitative assessment of starch grains before and after the clean-up procedure showed that >90% of the starch had been removed and further purification was felt to be unrewarding, and might lead to further degradation of residual labelled cellulose. Microscopic examination of the purified cellulose showed that the preparation retained significant aspects of tissue morphology, parenchymatous skeletons, and structural fibres were clearly visible. Aqueous solubility studies on this purified cellulose showed that less than 1% was soluble and filtration studies showed that greater than 98% was retained by a $0.45\ \mu$ Millipore filter. This product was used for human digestion studies.

In further purification experiments, dimethyl sulphoxide was used to solubilise the contaminating starch followed by enzymic hydrolysis as described above. This preparation showed identical solubility and microscopical characteristics to the purified cellulose used for human studies.

FAECAL ANALYSIS

Frozen faecal samples were examined radiographically and the number of radio-opaque pellets counted. The samples were then thawed and homogenised with an equal volume of water. Multiple aliquots (1 g) of the faecal homogenate were combusted in a Packard Model 306 sample oxidiser and total released $^{14}\text{CO}_2$ collected. The $^{14}\text{CO}_2$ was quantified using a Packard Model 3375 liquid scintillation counter with external standard channel ratio correction for quenching. Using this procedure, the recovery of ^{14}C -cellulose added to 1 g faecal samples *in vitro* was 95–106%. Faecal radioactivity in individual samples was expressed as a percentage of the dose administered and cumulative faecal excretion calculated.

The proportion of soluble faecal radioactivity was determined by centrifuging aliquots of the faecal homogenate at 25 000 G/30 minutes followed by filtration (Millipore $0.45\ \mu$ filter).

EXPIRED AIR $^{14}\text{CO}_2$

The samples collected directly into scintillation vials

were counted in a Packard Model 3375 liquid scintillation counter using Fisofluor I (Fisons Scientific Apparatus Ltd) as scintillant. The results are expressed as dpm/mmol CO₂ and cumulative excretion expressed as the percentage of the dose administered assuming a CO₂ excretion of 9 mmol/kg body weight/hour.⁸

Results

The overall recovery of radio-opaque pellets was 85% or greater (Table 1) in all subjects except one (subject no 3), who subsequently admitted the loss of one early faecal sample. The faecal results from this subject have been excluded. In the remaining nine subjects, of the 216 pellets administered, 198 were recovered in the faecal samples, a mean recovery of 92% (range 85–100%).

The recovery of faecal ¹⁴C and of expired air ¹⁴CO₂ in the subjects studied is shown in Table 1. The mean cumulative percentage faecal excretion of administered ¹⁴C-cellulose was 57.2±13.3 (1SD). The faecal recovery was lower in the older subjects (mean 52.0±7.5) than in the younger subjects (mean 63.8±16.6) but did not reach statistical significance. The faecal recovery of ¹⁴C has not been corrected for radio-opaque marker recovery.

The proportion of total faecal ¹⁴C soluble in the aqueous fraction of faecal material varied from 2.6–14% (mean 7.5±4.4 1SD). There was no correlation between total faecal ¹⁴C activity and the proportion soluble (r=0.475, p>0.05).

The transit time defined as that time at which 80% of radio-opaque pellets were recovered varied from

24–80 hours in the subjects studied, and did not correlate with the total recovery of faecal radioactivity (r=0.06, p>0.05). There was, however, a close correlation (Fig. 1) between the percentages of administered radioactivity and radio-opaque pellets present in individual faecal samples (r=0.90, p<0.001). In the nine subjects for whom complete faecal collections were available, a mean of 24% of the administered dose was excreted within the first 24 hours, 18% between 24 and 48 hours, 14% between 48 and 72 hours and 7% between 72 and 96 hours.

The pattern of expired air ¹⁴CO₂ excretion was similar in all subjects and a typical example is shown in Figure 2. An early sharp peak of breath CO₂ (mean time 1.55 hours, range one to three hours) was followed by a much broader second peak occurring between 10 and 30 hours. Significant radioactivity could be detected in expired air as early as 30 minutes after cellulose administration and continued for a minimum of 72 hours in all subjects. The cumulative excretion of breath ¹⁴CO₂ varied from 7.6–32.2% of the administered radioactivity and did not correlate with the total faecal excretion of ¹⁴C (r=0.515, p>0.05). The mean excretion in the elderly subjects, 22.2±7.5 1SD% was significantly higher (t=2.63, p<0.05) than in the younger subjects, mean 10.5±1.7 1SD (Table 2). The percentage of the oral cellulose excreted at various intervals after dosage is shown in Table 2.

Table 1 Age and sex of subjects studied. Cumulative excretion of radio-opaque pellets, ¹⁴C in faeces and ¹⁴CO₂ in expired air is expressed as a percentage of the cellulose oral dose. The faecal soluble ¹⁴C is expressed as a percentage of the total faecal ¹⁴C output.

Subject no	Age	Cumulative radio-opaque pellets % dose	Faecal recovery		Cumulative expired air ¹⁴ CO ₂ % dose
			Total ¹⁴ C % dose	% ¹⁴ C Soluble	
1	80	85	52	3.8	ND
2	82	92	48	2.6	ND
3	79	50	ND	ND	14.1
4	79	100	48	14.0	22.7
5	81	96	65	13.0	19.8
6	79	88	47	3.6	32.2
7	50	96	80	8.0	7.6
8	39	100	76	12.0	15.2
9	47	88	51	5.5	8.6
10	42	85	48	5.0	10.6
Mean±1SD		92±6.0	57.2±13.3	7.5±4.4	16.35±8.3

ND=not done in these patients.

Fig 1 Relationship of faecal excretion of radio-opaque pellets and ¹⁴C radioactivity after their simultaneous administration in 10 subjects. Each point represents a single faecal sample.

At all time intervals except 0–4 hours the expired air $^{14}\text{CO}_2$ is higher in the elderly subjects and is significantly higher at 24–48 hours.

Discussion

In a recent paper using an iodinated (I^{131}) cellulose which retained the physical and chemical characteristics of this class of fibre and which was resistant to acid peptic digestion, a mean of 87% of an oral dose was recovered in faeces.⁹ Of the I^{131} label appearing in the faeces less than 2% was filterable through a Millipore 0.45 μ filter. This study, though not primarily designed to study cellulose digestion, does suggest that orally ingested cellulose is, to a large extent, undigested and excreted in the faeces. The present study supports these results to some extent; in our younger subjects greater than 60% of an oral dose of C^{14} labelled cellulose is excreted in the faeces though in older subjects the faecal excretion is somewhat less. Our results differ from those of Carryer *et al*⁹ in that a significant proportion of the faecal radioactivity is filterable through a 0.45 μ filter, suggesting that some cellulose is degraded before excretion in the faeces. The I^{131} -labelled cellulose used by Carryer *et al*⁹ was fed in the form of long strands measuring 1–5 mm in length, and the discrepancy between the present results and theirs may reflect the difference in physical form of the two cellulose preparations. The present results give no indication of the nature of these non-absorbed degradation products.

It is assumed in man that the major site of non-starch polysaccharide breakdown is the colon, though the available information is scanty. As human digestive juices do not contain cellulases^{10 11} the digestion of cellulose in man presumably results from bacterial action, particularly in the colon. Holloway *et al*¹¹ using patients with ileostomies

Fig 2 Pattern of appearance of $^{14}\text{CO}_2$ in expired air in one subject after oral administration of ^{14}C -cellulose.

showed that over 80% of a mixed dietary source of cellulose was recovered in the ileostomy effluent in contrast with just over 20% in the faeces of control subjects. It should be emphasised that this study showed very wide variation in cellulose digestion in ileostomy patients and the methods used for assay have been questioned.¹ The early appearance of $^{14}\text{CO}_2$ in the expired air of our patients suggests either that a proportion of the cellulose suspended in the liquid phase reached the caecum early, or that degradation occurred in the small intestine. Bacterial action in the large bowel is probably the likeliest explanation. We cannot, however, exclude the possibility that the cellulose preparation used contains a proportion of residual non-cellulose polysaccharide which is responsible for this early peak. This possibility is unlikely as the cellulose preparation has been extracted by established and recommended procedures.⁵ The later peak of expired air $^{14}\text{CO}_2$ presumably results from colonic bacterial digestion. This peak is quantitatively much greater than the early peak, thus emphasising the major role of colonic bacteria in cellulose digestion.

Table 2 Expired air $^{14}\text{CO}_2$ as a percentage of administered dose at intervals after an oral dose of ^{14}C -cellulose in four elderly and four younger control subjects

Subject no	Age	0–4 h	4–10 h	10–24 h	24–48 h	48–72 h	Cumulative 0–72 h
1	78	2.6	4.9	7.5	6.3	1.4	22.7
2	79	1.2	2.1	8.2	7.6	0.7	19.8
3	80	0.8	1.4	6.1	5.8	0	14.1
4	79	1.6	3.0	10.2	11.9	5.5	32.2
Mean $\pm$ 1SD		1.56 $\pm$ 0.77	2.85 $\pm$ 1.52	8.0 $\pm$ 1.71	7.9 $\pm$ 2.78	1.90 $\pm$ 2.47	22.22 $\pm$ 7.53
5	50	1.9	1.6	3.4	0.7	0	7.6
6	39	1.3	1.4	3.7	4.2	0	10.6
7	47	1.7	2.2	7.9	3.4	0	15.2
8	42	2.6	1.6	3.4	1.0	0	8.6
Mean $\pm$ 1SD		1.88 $\pm$ 0.27	1.70 $\pm$ 0.35	4.6 $\pm$ 2.20	2.32 $\pm$ 1.74*	0	10.5 $\pm$ 1.69*

* Significantly lower ($p < 0.02$) than older group.

In earlier unpublished studies we have also shown that colonic sterilisation virtually abolished the later peak of expired air $^{14}\text{CO}_2$ without influencing the early peak.

The majority of studies in man on cellulose digestion have used a mixed dietary source of cellulose. The results of our studies can only be interpreted in relation to the type of cellulose used and further work will be required to establish whether these results apply to cellulose from different sources. One of the features of reports up to now on cellulose digestion in man has been the extreme variability in apparent digestibility found. This has been attributed to differing microbial flora,² to the source of the cellulose as well as its degree of purification,¹ and to inadequate methodology. The variation in fermentation of different celluloses by cellulolytic organisms has also been stressed.¹² The cellulose used in the present studies has been prepared from leafy material and has been further purified to remove non-cellulose polysaccharides. The variation of previous reports on the apparent digestibility of cellulose in man makes comparison with our results difficult to assess. The results of Southgate and Durnin² and of Milton-Thompson and Lewis,⁴ both of whom used mixed dietary cellulose, would approximate to our results, but the more recent results of Cummings *et al.*¹³ and Prynne and Southgate³ reported considerably higher digestibility. It should be emphasised, however, that most of these studies using a mixed dietary source of cellulose showed considerable individual variation in apparent digestibility. It is suggested that more purified celluloses are less readily digested¹² though information available in man is limited. Differing methodology must also be considered, and as a proportion of the cellulose, though digested, is excreted in the faeces as an aqueous soluble product, this may not be included in chemical assays of faecal cellulose. Furthermore, the insoluble fraction in faeces may also be altered by bacterial metabolism and not be included in chemical assays. A great deal of further work needs to be performed to identify the chemical nature of the labelled cellulose metabolites excreted in the faeces of our subjects.

It is unlikely that cellulose, or dietary fibre in general, is an important nutritional component of human diets. Volatile fatty acids are a major product of microbial degradation of fibre and the major portion of these volatile fatty acids is absorbed¹⁴ and used as an energy source. Our studies show that up to 38% of ingested cellulose may be digested, absorbed, metabolised, and eventually appear in the expired air as CO_2 . The mean excretion of CO_2 from ingested cellulose in

our patients amounted to 18%. The mean cumulative excretion of ^{14}C in faeces and expired air accounted for 74% of the dose administered. The remaining 26% not accounted for can be explained by a combination of incomplete faecal collection as only 90% of radio-opaque pellets were recovered, late excretion of $^{14}\text{CO}_2$ in expired air after 72 hours, $^{14}\text{CO}_2$ at low levels was detected in some patients for up to seven days after the dose was administered, and perhaps small quantities lost in flatus or faecal gas, urine, and body pool. Overall, the energy value of dietary fibre in western man is probably quite low.¹ Much further work will be required to clarify the physiological and biological effects of bacterial breakdown of cellulose and other dietary fibres within the human gastrointestinal tract.

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