Effect of in vitro fermentation using human faecal inoculum on the water-holding capacity of dietary fibre

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1. The water-holding capacities (WHC) of four sources of fibre were measured using dialysis membranes and osmotic-suction pressures of 45, 89 and 178 mosmol/l (1, 2 and 4 atm). At all pressures, pectin had the highest WHC, followed by cabbage (*Brassica oleracea*) and lucerne (*Medicago sativa*) and then cellulose. A suction pressure of 89 mosmol/l (2 atm) was used in the subsequent fermentation study since it had the lowest standard error of the mean and most closely approximated physiological conditions.

2. The four fibres were anaerobically fermented in vitro with human faecal inoculum for 24 h. The WHC of the fermentation residues were measured. The potential water-holding capacity (PWHC), a function of the extent of fermentability and the WHC of the fermentation residues, was highest for lucerne, followed by cellulose, then cabbage and, finally, pectin. Only the PWHC values ranked the four fibres in the same order as in vivo values.

3. It was concluded that the ethanol-insoluble residues containing unfermented fibre organic matter and microbial organic matter, both of which hold water, should be used to calculate PWHC and to predict the effect of fibre on rate of passage and faecal mass in humans.

The epidemiology of colonic diseases suggests that the incidence may result from an inadequate intake of dietary fibre (Burkitt *et al.* 1972). Many of the beneficial effects of dietary fibre seem to be related to its ability to increase faecal output from the gastrointestinal tract of man and the rate of passage through it (Van Dokkum *et al.* 1983). The bulk of the undigested food is related to passage and it is thought that the capacity of the fibre to retain water is important (McConnell *et al.* 1974; Brodribb & Groves, 1978; Robertson *et al.* 1980). This explanation is inadequate, however, since neither the fermentability of the fibre source, nor the concomitant microbial proliferation is considered. Moreover, it has been found that the ingestion of dietary fibres that are very high in water-holding capacity (WHC) does not seem to alter mouth-to-anus rates of passage in humans (Van Soest *et al.* 1978). In fact, Stephen & Cummings (1979) observed an inverse relationship between WHC and stool weight.

These discrepancies between the proposed mechanism and in vivo information are found primarily with water-soluble polysaccharides and vegetable fibres, both of which are rapidly and completely fermented. Elucidation of the role of dietary fibre in large-bowel diseases will require an understanding of the substrate composition, rate and extent of fermentability, WHC, and the mass of microbes proliferating in the gut.

The purpose of the present study was to examine the effect of 24 h in vitro fermentation by human micro-organisms on the WHC of four dietary fibres. The study provides evidence that the 24 h extent of substrate fermentation and the mass of micro-organisms produced are important aspects in determining the mass of human faeces and its WHC.

EXPERIMENTAL

Materials

The following dietary fibres were studied: lucerne (*Medicago sativa*; neutral-detergent fibre, NDF), cabbage (*Brassica oleracea*; ethanol-insoluble matter, EIM), pectin and wood

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cellulose. These fibres represented the range of fermentability and WHC encountered in the human diet. It was necessary to extract the dietary fibres of lucerne and cabbage to compare them directly with pectin and cellulose, free of interference from the soluble contents of plant cells. Consequently, the dietary fibres of lucerne and cabbage are not in the form naturally eaten, but in a form in which the contribution of the physical properties of the respective fibres could be measured. The organic matter (OM), EIM and NDF yields (g) from 1 g unextracted food dry matter (DM) were 0·928, 0·729, 0·530 for lucerne; 0·911, 0·426, 0·142 for cabbage; 0·971, 0·971, 0·005 for pectin; and 0·996, 0·996, 0·996 for cellulose respectively. The lucerne was prepared by amylase (*EC* 3.2.1.1 and 3.2.1.2) digestion and neutral-detergent extraction to remove interferences from starch and protein. Lucerne is high in lignin and cellulose, similar to wheat bran, and has been used in many in vivo studies. The cabbage was converted to a coarse powder by marinating in ethanol (800 ml/l), passing the alcohol slurry through a meat grinder, and extracting twice more with ethanol (950 ml/l) followed by acetone. This treatment concentrated the dietary fibre thirty-fold relative to the raw cabbage and resulted in material similar to that used in a previous in vivo study (Van Soest *et al.* 1978). The pectin (No. P-9135; Sigma Chemical Co., St Louis, MO) was the methyl ester of polygalacturonic acid. The cellulose (Solka floc; SW-40, Brown and Co., Berlin, NH) was used as purchased.

The DM and OM contents of each sample were determined in duplicate by drying overnight at 100°, hot-weighing and then ashing (Goering & Van Soest, 1970). The NDF was determined by the modified detergent method (Robertson & Van Soest, 1981). The EIM content was measured by weighing 1 g of the sample into a beaker, adding 250 ml ethanol (800 ml/l) and covering the beaker with Parafilm. After 24 h at room temperature, the EIM was gravity-filtered through a preweighed filter paper and washed with ethanol (800 ml/l). The DM and OM contents of the EIM were determined in the same manner as the whole sample.

WHC method

The WHC of a sample was measured by weighing the hydrated sample after subjecting it to osmotic suction across a dialysis membrane (Robertson & Eastwood, 1981*a*, *b*). The sample was dried at 100° and reweighed to determine the amount of water held per g sample.

Dialysis tubing with a molecular weight cut-off of 2000 (Spectropor 6; 45 mm, no. 132630; VWR Scientific, Rochester, NY) was cut into 100-mm lengths and was soaked overnight in a sodium azide solution (1 ml/l). One end of the dialysis tubing was tied tightly with waxed dental floss and then 100 mg of the sample was placed into the tubing, followed by 5 ml sodium azide solution to hydrate the sample. The other end of the tubing was tied off before placing the dialysis bag into a 150 ml beaker. The beakers were placed inside a desiccator with water in the bottom to maintain a high relative humidity and to prevent water evaporation. After 24 h, 50 ml of a polyethylene glycol (PEG) solution was pipetted into each 150 ml beaker. Suction pressures of 45, 89 and 178 mosmol/l (1, 2 and 4 atm) were produced by adding 64, 95 and 138 g, respectively, of PEG (molecular weight 3350, no. P-3640; Sigma Chemical Co.) per litre sodium azide solution. The osmolarity was determined with a freezing-point-depression osmometer. After 72 h the desiccator was opened, the dialysis bag was removed and cut open. The sample was transferred from the dialysis bag into a dry, weighed test-tube (wtt). The test-tube and hydrated sample were weighed immediately (wh) and again after overnight drying (wd) at 100°. The difference in weight represented the water held by the sample against the suction pressure (wh − wd). The weight differences between the empty test-tube and test-tube with the dry sample represented the DM (wd − wtt). The weight was corrected to an OM basis (wo = (wd − wtt) × % OM). The WHC is expressed as g water held per g OM as follows:

$$\text{WHC} = \text{g water/g OM} = (\text{wh} - \text{wd})/\text{wo}.$$

Fermentation method

A batch-culture technique utilizing human faecal microflora was used to measure 24 h in vitro fermentability of the substrates and to obtain fermentation residues (Jeraci, 1981). A healthy, 26-year-old male provided the inoculum. Anaerobiosis was maintained throughout the collecting, processing, and inoculating periods. The culture medium and fermentability assay were the same as for the rumen in vitro procedure (Goering & Van Soest, 1970).

Isolation of fermentation residues for WHC

Neutral-detergent residues (NDR) were obtained by extracting the contents of duplicate in vitro fermentation flasks with 100 ml refluxing neutral-detergent solution for 1 h and filtering (Robertson & Van Soest, 1981). Contents from other duplicate flasks were transferred to 600-ml beakers, adjusted to 800 ml ethanol/l with 950 ml ethanol/l, covered with Parafilm, allowed to settle for 24 h, and gravity-filtered through filter paper to isolate the ethanol-insoluble residue (EIR). Total residues (TR) were obtained by agitating the contents of similar in vitro flasks for 1 min before removing 6 ml for analysis.

Determination of extent of fermentation

The NDR was obtained in the same manner as the neutral-detergent isolate, but was dried at 100° to determine the amount of DM remaining. The weight of the EIR was determined by filtering the residue through filter paper that had been weighed after drying at 100° and then weighing the EIR and filter paper after drying. The DM of the TR was measured by removing 21·0 ml from the in vitro flask during agitation. The slurry was placed in a preweighed beaker, dried at 100° and weighed. All the residues were then ashed at 550° and weighed again; the OM content was determined by difference. The EIR and TR both contained precipitated salts from the in vitro media which confounded all calculations, including DM, so adjustment to an OM basis was necessary. Duplicates were done for each residue, substrate and time.

The additional OM contributed by the inoculum was included in the calculation for both unfermented EIR and unfermented TR by adding in the OM of the zero time control.

$$\text{Unfermented EIR (and TR)} = \frac{\text{24 h residue OM}}{\text{(original substrate OM} + \text{0 time OM)}}.$$

Since the inoculum did not contain any measurable NDF, the adjustment was not necessary for the unfermented NDR calculations.

WHC of fermentation residues

The WHC of the fermentation residues were measured in the same manner as the original substrates with equilibrium at 89 mosmol/l (2 atm). The WHC of TR was measured by pipetting 6 ml directly from the in vitro fermentation flask into the dialysis bag. The WHC of the residues was calculated as g water/g organic residue.

The potential WHC (PWHC) was calculated as g water held by the fermentation residue from 1 g original substrate.

$$\text{PWHC} = \text{(fermentation residue)} \times \text{(WHC of residue)},$$

and has the units of g water/g original substrate OM. The PWHC is a function of the amount of residue remaining and the altered WHC of the residue.

Statistics

The results were tested for significance using the Student's t test for equal and unequal sample sizes (Snedecor & Cochran, 1976).

Table 1. *Water-holding capacity (WHC) of four sources of fibre measured at three osmotic suction pressures*

(Values are means of triplicate determinations with their standard errors)

	WHC (g water/g organic matter)					
Suction pressure (mosmol/l)...	45		89		178	
(atm)...	1		2		4	
Source of fibre	Mean	SEM	Mean	SEM	Mean	SEM
Lucerne (*Medicago sativa*)	2·18	0·07	1·57	0·07	1·50	0·11
Cellulose	0·67	0·01	0·64	0·07	0·64	0·17
Cabbage (*Brassica oleracea*)	3·53	0·50	2·80	0·33	4·17	0·63
Pectin	15·81	1·24	10·96	0·13	6·89	0·17

Table 2. *Neutral-detergent residue (NDR), ethanol-insoluble residue (EIR) and total residue (TR) remaining from 1 g neutral-detergent fibre (NDF), ethanol-insoluble matter (EIM) and organic matter (OM) of four sources of fibre fermented in vitro with human faecal inoculum for 24 h*

(Values are the average of duplicate determinations)

	g NDR	g EIR	g TR
Source of fibre	g NDF	g EIM	g OM
Lucerne (*Medicago sativa*)	0·808	0·807	0·698
Cellulose	0·779	0·797	0·805
Cabbage (*Brassica olearacea*)	0·106	0·364	0·603
Pectin	0·000	0·220	0·506

RESULTS

All of the OM of the four substrates was insoluble in 800 ml ethanol/l. Pectin and cabbage were extensively soluble in neutral-detergent solution.

WHC of the four sources of fibre

The WHC of the substrates measured at three osmotic suction pressures are shown in Table 1. At all pressures, the WHC were: pectin > cabbage > lucerne > cellulose. The lowest standard error of the mean (SEM) was obtained at 89 mosmol/l (2 atm) for pectin, cabbage and lucerne. The SEM of the cellulose WHC was especially low at all pressures. The PWHC of all the residues from lucerne, cabbage and pectin were different from the WHC of the original materials ($P < 0·05$).

Extent of fermentation at 24 h

The OM of the NDR, EIR and TR remaining after 24 h of fermentation per g OM at zero time are shown in Table 2. Pectin and cabbage had similar amounts of NDR and EIR as did the lucerne and cellulose. The TR was different for all samples. The TR was composed of the unfermented substrate, the microbial mass and the low-molecular-weight OM. The pectin and cabbage TR, because of their extensive fermentation, contained more microbial

Table 3. *Water-holding capacity (WHC) of residues of four sources of fibre fermented in vitro with human faecal inoculum for 24 h and measured at an osmotic pressure of 89 mosmol/l (2 atm)*

(Values are means of duplicate determinations with their standard errors)

	WHC (g water/g residue)					
	Neutral-detergent		Ethanol-insoluble		Total	
Source of fibre	Mean	SEM	Mean	SEM	Mean	SEM
Lucerne (*Medicago sativa*)	1·26*	0·06	1·44	0·23	0·94**	0·08
Cellulose	0·64	0·05	0·99**	0·11	0·65	0·02
Cabbage (*Brassica oleracea*)	0·40**	0·01	2·31*	0·19	—	—
Pectin	—	—	1·75**	0·30	2·55**	0·37

Mean values were significantly different from the corresponding original substrate: $*P < 0·1$, $**P < 0·05$.

mass and end-products which increased the amount of TR. Some of these compounds may be absorbed from the gastrointestinal tract. Therefore, TR values overestimate the mass that would remain in the gut, which is in agreement with the findings of Cummings *et al.* (1979) and Holloway *et al.* (1983).

EIR included the microbial mass and the unfermented high-molecular-weight OM of the substrate. The microbial mass is a significant portion of the faecal matter (Stephen & Cummings, 1980) and could be important in terms of WHC. The NDR recovers the unfermented fibre but does not contain microbial matter or water-soluble polysaccharides; thus, NDR underestimates the mass remaining in the lower gut. However, the NDR does yield the most accurate estimate of digestibility of the fibre substrate.

WHC of the fermentation residues

The WHC of the in vitro residues after 24 h fermentation are shown in Table 3. Pectin did not have an NDR so WHC could not be measured. The WHC of the cabbage TR could not be determined because a mucilaginous film coated the dialysis membrane during the 72 h equilibration.

Fermentation reduced the WHC of the lucerne from 1·57 to 1·26 g water/g OM. The WHC of the lucerne NDR was significantly decreased by fermentation. The original lucerne substrate was a neutral-detergent isolate so the WHC of the unfermented lucerne and the lucerne NDR are comparable. The similarity of the WHC of the lucerne EIR and original lucerne substrate may be due to the microbial contribution to the EIR WHC. The WHC of the lucerne TR was different from the unfermented lucerne, probably due to the low-molecular-weight compounds contained in the TR.

Only the EIR of the cellulose had a higher WHC than the original material. As with the lucerne, the microbial mass produced in the fermentation and a concomitant decrease in the extent of cellulose crystallinity increased the WHC of the residue.

The WHC of the cabbage NDR and EIR were both less than for the unfermented cabbage. It was not possible to determine if the cabbage residue had a decrease in WHC due to fermentation since the cabbage EIR recovered all the remaining cabbage substrate as well as the microbial mass, which may have a lower WHC than the residual cabbage. This would lower the WHC of the cabbage EIR.

Pectin fermentation residues (EIR and TR) had a very low WHC when compared with the unfermented pectin. Pectin was almost completely fermented, as measured by uronic

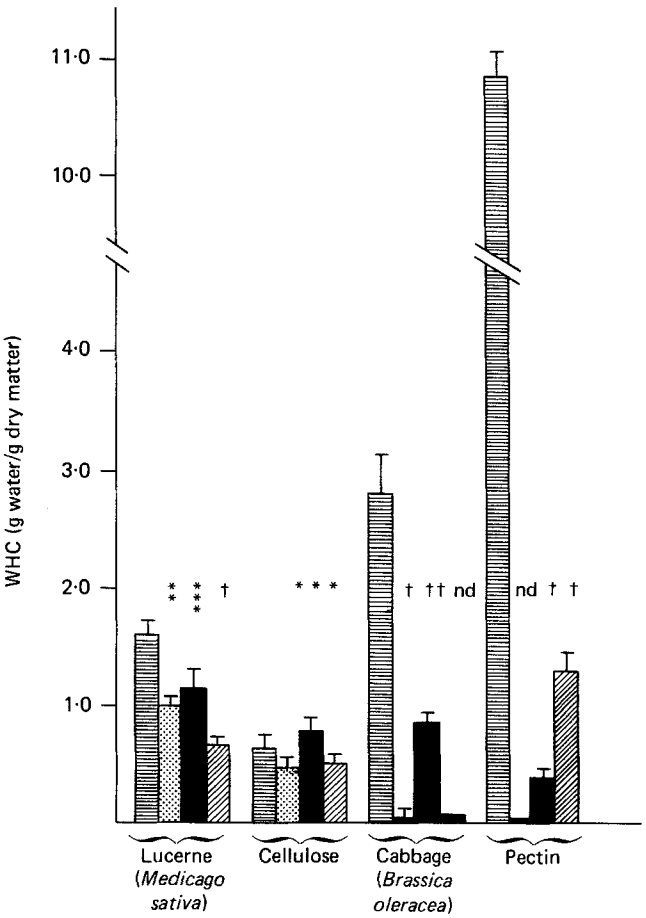

Fig. 1. Potential water-holding capacity (WHC) of three residues ((▩), neutral-detergent residue; (■), ethanol-insoluble residue; (▨), total residue) from human in vitro fermentation and the WHC of the original substrate (▤) measured by osmotic suction pressure of 89 mosmol/l (2 atm). Values are means with their standard errors represented by vertical bars. Mean potential WHC values were significantly different from the WHC of the corresponding original substrate: $*P < 0{\cdot}01$, $**P < 0{\cdot}02$, $***P < 0{\cdot}05$, $\dagger P < 0{\cdot}001$, $\dagger\dagger P < 0{\cdot}002$; nd, not determined.

acid units (Horvath, 1984). Consequently, the pectin EIR and TR consisted primarily of microbial OM having an estimated WHC of 2 g water/g OM.

The PWHC of the three fermentation residues and the WHC of the corresponding original substrates are shown in Fig. 1. The PWHC accounted for the proportion of the ingested fibre that would be lost through fermentation and could not contribute to the WHC of the gut contents except through microbial mass. PWHC places all the residues on an equal basis with the ingested (or unfermented material) by considering the extent of fermentation. The PWHC differed in magnitude from those of the WHC of the residues only for the cabbage and pectin. Lucerne had an unfermented WHC greater than the PWHC of each of its residues.

DISCUSSION

Three different suction pressures were evaluated using the technique of Robertson & Eastwood (1981*a*) to determine the WHC of water-soluble polysaccharides. The method was modified so that the amount of water held at each pressure was estimated by drying the hydrated substrate and using the weight loss as a measure of bound water. A review of the literature indicated that an osmotic difference of approximately 89 mosmol/l (2 atm) is present across the colonic lining (Phillips & Giller, 1973; Goodhart & Shils, 1980; Mailman, 1981). This suction pressure gave the lowest error in the present study. Therefore, a suction pressure of 89 mosmol/l (2 atm) was used for the subsequent measurements.

Previous studies had implied that a loss of fibre, the presence of fermentation products (Williams & Olmstead, 1936), or a change in the composition and WHC of the fibre might explain the poor correlation of wet stool weight and rate of passage by WHC (McConnell *et al.* 1974; Stasse-Wolthuis *et al.* 1980; Kay, 1982). Stephen & Cummings (1979) reported an inverse relationship between wet stool weight and the WHC of the ingested material. This could be explained if fibres of high WHC also had a greater degree of fermentation. Indeed, in the present study, the fibres that held more water did ferment to a greater extent. Only the neutral-detergent fraction of lucerne had a lower WHC after fermentation. The WHC of the NDF, EIM and OM allows one to distinguish the effects of different fibre sources on effective upper-intestinal-tract, prefermentative WHC; rate of passage (particle size); and rate of absorption of soluble entities in the diet. However, the NDR, EIR and TR recovered the unfermented fibre, the microbial OM and the soluble organic components to different degrees as previously discussed in this paper. Consequently, the NDR, EIR and TR represent different fractions of the caecal and colonic contents and the WHC measures the contribution of these residues to stool wetness and bulk.

Four factors that might alter wet stool weight are: (1) the change in WHC of the fibre remaining after fermentation, (2) the loss of OM, (3) the addition of microbial mass, (4) particle size. Particle size was not a variable in the present study. Analysis of the NDR, EIR and TR confirmed the hypothesis that the first three factors are important. The significantly lower WHC of the lucerne NDR compared with the WHC of the lucerne NDF provided evidence that fermentation directly reduced the WHC of fibre since microbial OM was removed by the neutral-detergent procedure. The cellulose EIR had a higher WHC than the original cellulose suggesting that the microbial OM generated from the fermentation of cellulose had a higher WHC than did the cellulose that was fermented. It was impossible to determine if the cabbage OM lost WHC due to fermentation since some of the EIM which is soluble in neutral-detergent solution may not have been fermented. Only the cabbage EIR recovered all the unfermented cabbage OM, but the question could not be resolved since the cabbage EIR contained microbial OM. However, the observation that the WHC of the cabbage EIR was less than the EIM suggests that the microbial OM had a lower WHC than did the cabbage that was fermented by the microbes. Therefore, the amount of fibre fermented and its ability to hold water must be considered as well as the amount of microbial OM produced if one is to predict the effect of fibre on colonic contents. For example, the pectin originally had a high WHC, but it was completely fermented. Consequently, the stool bulk would result primarily from microbial OM.

The PWHC estimates of three different residues (NDR, EIR and TR) were statistically different from the WHC of the corresponding original substrates. The different residues showed a range of PWHC values relevant to the physiological effect of fibre in the gut. Potential WHC is a measure of the relative WHC that equivalent amounts of ingested fibre have in the colon. The PWHC of the EIR is the most meaningful since EIR contains most of the unfermented substrate OM and the microbial mass. The WHC of the ingested

material may have significance in predicting the transit time from mouth to colon. However, fermentation must be considered to predict colonic mass and rate of passage.

In the present study, only the PWHC values ranked sources of fibre in the same order as have in vivo studies (McConnell *et al.* 1974; Stasse-Wolthuis *et al.* 1980). The PWHC measurements showed an inverse ordering of the four original substrates relative to the original substrates. Using PWHC instead of the WHC of original substrates reverses the relationship seen by Stephen & Cummings (1979), resulting in better predictions of stool weight and rate of passage. The preferred residue to use for PWHC estimates is the EIR since both the unfermented fibre OM and microbial mass are included.

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