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Session: Physiological aspects of fibre

The active fraction of psyllium seed husk

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A series of experiments and evaluations of fractions isolated from psyllium seed husk (PSH) were used to test the overall hypothesis that a gel-forming component of PSH is not fermented and that it is this component that is responsible for the laxative and cholesterol-lowering properties of PSH. A gel is isolated from human stools collected during a controlled diet study when PSH is consumed but not when the control diet only is consumed. Evaluations of three fractions isolated from PSH suggest that gel-forming fraction B, which is about 55 % of PSH, is poorly fermented and is the component that increases stool moisture and faecal bile acid excretion, the latter leading to lower blood cholesterol levels. Fraction C, representing < 15 % of PSH, is viscous, but is rapidly fermented. Fraction A is alkali-insoluble material that is not fermented. In concentrations comparable with their presence in PSH, fractions A and C do not alter moisture and bile acid output. The active fraction of PSH is a highly-branched arabinoxylan consisting of a xylose backbone and arabinose- and xylose-containing side chains. In contrast to arabinoxylans in cereal grains that are extensively fermented, PSH possesses a structural feature, as yet unidentified, that hinders its fermentation by typical colonic microflora.

Psyllium seed husk: Dietary fibre: Laxation: Hypocholesterolaemia

Psyllium seed husk (PSH) has a long and established record as a bowel regulator (Cummings, 1993). More recently, it has been demonstrated to lower blood cholesterol levels (Anderson *et al*. 2000). These physiological properties have been thought to be due to the extraordinary gel-forming characteristic of this material. Over 50 years ago Laidlaw & Percival (1950) studied the chemical features of some fractions of the whole seed, and in the late 1970s Kennedy *et al*. (1979) reported some structural features of the carbohydrate extracted from the husk. In the 1990s Marteau *et al*. (1994) attempted to isolate a gel from stools of human subjects who had consumed PSH.

Our interest in psyllium began with the observation that a gel was present in stools of rats fed PSH (Cabotaje *et al*. 1994). This observation was obviously not an original one, but was so exceptional that our interest in this material was aroused. A series of experiments were initiated that had as their overall hypothesis that a gel-forming carbohydrate component of PSH is not fermented and it is this component that is responsible for the hypocholesterolaemic and laxative properties of PSH. These experiments are summarized in the present paper.

A controlled-diet study was conducted to obtain stools from subjects consuming PSH (Marlett *et al*. 2000). Fourteen healthy men and women (18–30 years old) completed the study that tested a dose of 8·8 g PSH as 15 g Metamucil®/d. The first phase of the study, a 12 d screening phase in which subjects consumed the supplement along with their usual diet, was used to evaluate their habitual diets and compliance with an experimental protocol. A controlled diet was consumed and the supplement was continued during the next 7 d, which was phase 2. This phase was followed by a 2-week washout period (phase 3) in which subjects consumed their usual diets. The final phase consisted of the same controlled diet as that consumed in phase 2, but without the fibre supplement. The responses to the fibre supplement were similar to those reported by Marteau *et al*. (1994; Table 1). Stool moisture and wet and dry weights increased markedly. Marteau *et al*. (1994) also reported that gastrointestinal transit time was not affected by

Abbreviation: PSH, psyllium seed husk.
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Table 1. Recent observations of large-bowel responses to psyllium seed husk (PSH)

Response	Marteau *et al.* (1994)	Marlett *et al.* (2000)
Subjects (*n*)	7	14
Study days: –PSH	15	7
+PSH	15	7
Dose of PSH (g/d)	18	8·8
Wet stool weight (g/d): –PSH	122	120
+PSH	231	190
Stool moisture (%): –PSH	69	74
+PSH	77	80

Table 2. Sugar composition (mg/g) of psyllium seed husk (PSH) and fractions (modified from Marlett & Fischer, 2002)

Sugar	PSH	Fr A	Fr B	Fr C
Arabinose	203	342	203	84
Galactose	41	129	16	17
Glucose	46	191	1	5
Mannose	22	100	tr	tr
Rhamnose	29	5	9	143
Uronic acids	54	35	20	245
Xylose	503	33	712	421

Fr, fraction; tr, <1 mg/g.

PSH in healthy adults, probably because it was within the normal range without the supplement, and thus unlikely to change (Harvey *et al*. 1973).

We observed that the psyllium-containing stool was excreted as a cohesive mass, but then lost shape after excretion and was gelatinous. As the material exhibited non-Newtonian behaviour during attempts to analyse it using a viscometer, this gelatinous characteristic was assessed by comparing the apparent viscosity of an aqueous extract of control and psyllium stools using an Ostwald dropping pipette viscometer. The apparent viscosity of the aqueous extract from the psyllium stool (mean 238 (SE 38) s) was significantly greater ($P < 0{\cdot}01$) than viscosity of the aqueous extract from the control stool (128 (SE 7) s).

Marteau *et al*. (1994) were unable to extract the gel from human stools using differing concentrations of ethanol. Our approach was to solubilise the fresh stool in alkali (Marlett *et al*. 2000). At this pH a negatively-charged environment is created in which the polysaccharide moieties repel one another, and the solution is no longer viscous. Insoluble material was then removed by centrifugation. The sample was acidified with acetic acid, and carbohydrate was precipitated with ethanol. This material was desiccated, rehydrated, heated rapidly to 100°C, cooled and insoluble material removed by centrifugation. The soluble fraction was concentrated and adjusted to 80 % (v/v) ethanol to recover a gel. A gel was isolated from the psyllium-containing stool, but not from the control stool, at a concentration of 75 (SE 1) mg/g dry stool weight. The majority of this isolated material was carbohydrate (763 (SE 18) mg sugar/g fraction) and most of the sugars were xylose (640 (SE 10) mg/g total sugars), and arabinose (270 (SE 0) mg/g total sugars), the same sugars that predominate in PSH (Table 2). This study is consistent with our hypothesis that a component of PSH is an unfermented gel that acts as an emollient to provide lubrication and facilitate propulsion of colon contents.

Using the same principles that were used to isolate the gel from stools, PSH was fractionated into three components (Marlett & Fischer, 2001, 2002). Fraction A was alkali-insoluble material and the yield was 171 (SE 4) mg/g; fraction B was gel-forming material and the yield was 575 (SE 16) mg/g; fraction C was a viscous, but not gel-forming material and the yield was 129 (SE 6) mg/g. We reported that PSH is almost all carbohydrate (902 mg/g), with small amounts of crude protein ($N \times 6{\cdot}25$; 35 mg/g) and ash (34 mg/g); the recovery of starting material was 971 mg/g. Most of the protein and ash is in fraction A, although fraction C, which is isolated last, contains ash, reflecting the extractants used in the isolation scheme. The sugar composition of PSH and the fractions, determined by GLC as alditol acetate derivatives, suggest that the fractions contain different polysaccharides (Table 2).

The ability of these fractions to alter ileal bile acid absorption was evaluated using the colectomized rat (Marlett & Fischer, 2002) in which the caecum and entire colon are surgically removed and the ileum is anastomosed to the rectum (Hildebrandt & Marlett, 1991). In this animal model faeces become soft-formed within 3–5 d post-operatively; pre-operative body weight is achieved within 6–10 d post-operatively, after which weight gain and food intake are not different from that of rats of comparable body weight (Hildebrandt & Marlett, 1991). We used this animal model because most of the microbial activity, which can modify up to half the bile acids in the large intestine (Tandon *et al*. 1984; Setchell *et al*. 1985), is eliminated. Animals were fed test meals by mouth that were nutritionally complete and marked with Cr_2O_3, and ileal excreta was collected for 24 h. PSH was fed at 50 mg/g test meal. Fractions were present in the test meals at the concentrations they were isolated from the husk, in order to evaluate their individual effectiveness. A cellulose (5 mg/g) test meal was the control diet. The total bile acid output in ileal excreta was ≥2-fold greater ($P < 0{\cdot}05$) when PSH and fraction B meals were fed (23 (SE 3) and 21 (SE 3) μmol/g test meal recovered respectively) than when fraction A, fraction C or cellulose meals were fed (11 (SE 1), 13 (SE 2) and 7 (SE 1) μmol/g test meal recovered respectively; Marlett & Fischer, 2002). The moisture content of the ileal excreta, which was 630–660 mg/g when fraction A, fraction C or the cellulose diet was fed, increased ($P < 0{\cdot}05$) to 860 and 900 mg/g respectively when fraction B and PSH meals were fed. The effects of fraction B, which was fed at 57 % of the concentration of the intact PSH, and the intact PSH on ileal excreta bile acid and moisture contents were not different. These findings support our hypothesis that fraction B is the physiologically-active component of PSH.

The hypothesis that a component of PSH was either not fermented or poorly fermented in the large intestine and thus could act as an emollient was evaluated using an *in vitro* fermentation system (Marlett & Fischer, 2002). Inoculum was prepared in an anaerobic chamber from caecal contents of rats fed a diet containing 50 mg PSH/g (Monsma & Marlett, 1995, 1996). Duplicate flasks containing about 2000 μmol

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carbohydrate were fermented for 0–72 h. The substrate consisted of veal infusion broth and yeast (providing 787 μmol carbohydrate) and PSH, one of the fractions, or the three fractions combined in proportion to their concentration in intact PSH. Control flasks containing only the veal infusion broth and yeast were also fermented. Fermentation was assessed by measuring the disappearance of total sugars, arabinose and xylose, and the production of short-chain fatty acids, all by GLC. Fraction A was essentially not fermented, with < 5 % of its constituent sugars disappearing at 48 h (Marlett & Fischer, 2002). Fraction C was rapidly fermented, with > 80 % of its constituent sugars disappearing in 24 h; no further fermentation was apparent at 48 h. Fraction B was poorly fermented, achieving about 25 % sugar disappearance at 72 h. Since fraction B represented over half the sugars in PSH and the combined fractions, these substrates also were poorly fermented, with about 35 % of the sugars disappearing at 72 h. Short-chain fatty acid production reflected these differing levels of sugar fermentation and the rapid fermentation of the veal infusion broth and yeast.

It is not known why the gel-forming component of PSH is not fermented by the microflora. Amounts of terminal arabinose, terminal xylose, 3,4-linked xylose, and 2,4-linked xylose in fraction B have been detected (Marlett & Fischer, 2001) that are different from those reported by Laidlaw & Percival (1950) and Kennedy *et al*. (1979). Our evidence (Marlett & Fischer, 2001) also indicates the presence of substantial amounts of 3-linked arabinose, which was not reported by the other investigators. Differences among the data from the three laboratories are not surprising as the polysaccharides studied by the three groups differed. Laidlaw & Percival (1950) determined the structural features of mucilaginous material isolated with hot water from whole seed that had been previously extracted with cold water. Kennedy *et al*. (1979) studied the carbohydrate in PSH by methylating the entire husk. We (Marlett & Fischer, 2001) have examined the carbohydrate isolated as fraction B. The earlier research indicated that the gel in PSH is a highly-branched arabinoxylan, in which xylose is the backbone, and arabinose and xylose form the side chains. Our studies confirm these findings. However, there is more to the story of non-fermentability; other arabinoxylans, such as those in wheat and oats, are extensively fermented (Chen *et al*. 1998). Interestingly, in our *in vitro* study arabinose was less fermented than xylose (Marlett & Fischer, 2002). Arabinose digestibility was also less than that of xylose in the human studies of PSH conducted by Marteau *et al*. (1994) and ourselves (Marlett *et al*. 2000). Thus, we speculate that the linear chain of PSH gel is fermented until an atypical branch point is reached that is not available to microbial activity.

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