# Composition of faeces from human subjects consuming diets based on conventional foods containing different kinds and amounts of dietary fibre

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**The stool-bulking effect of dietary fibre (DF) is well-documented and believed to be important in the postulated beneficial effect of DF on human health. The aim of the present study was to investigate the digestibility of DF in relation to its stool-bulking properties and to study possible mechanisms for this effect. Four diets, based on conventional foods only, were studied in balance experiments on human subjects. Diet A contained DF mainly from whole-grain cereals while diets $B_1$ and $B_2$ contained DF mainly from pulses, vegetables and fruit. Diet C was a low-fibre diet. Faeces was fractionated into four fractions, each enriched in one of the following three components: undigested DF (fractions 1 + 2), faecal bacteria (fraction 3) and soluble components (fraction 4). The digestibility of DF in diets A, $B_1$ and $B_2$ was 0·62, 0·88 and 0·90 respectively. Subjects consuming diet A excreted slightly more fraction 3 than subjects consuming the other diets. Thus, the statement that DF of high digestibility stimulates microbial growth in the gut was not supported. The water-holding capacity of fraction 1 was studied in vitro and was found to be low. It is suggested that undigested soluble DF is important in the stool-bulking properties of DF.**

**Bacteria: Dietary fibre: Faecal composition**

One of the most well-documented physiological effects of dietary fibre (DF) is its ability to increase stool output (Kelsay, 1978). Different kinds of DF have been shown to be effective to varying degrees in this respect and it has been suggested (Stephen & Cummings, 1980*a*) that more than one mechanism is responsible for this stool-bulking effect. However, suggested mechanisms have been formulated on the basis of results obtained in studies where concentrated fibre preparations, rather than conventional fibre-rich foods, were used. This limits conclusions relating to the validity of these mechanisms for diets where DF is derived from conventional foods. This is of interest since the dietary fibre hypothesis (Burkitt & Trowell, 1975) was based on information derived from populations consuming conventional foods rather than concentrated preparations of DF.

Stephen & Cummings (1980*a*) have presented evidence suggesting that DF of high digestibility increases faecal output by stimulation of microbial growth in the large intestine. Studies of the intestinal microflora are, however, difficult. Available techniques require sophisticated equipment and are expensive, time-consuming and not necessarily valid or accurate enough for studies of the relationship between dietary composition and intestinal microflora. To overcome these difficulties, Stephen & Cummings (1980*b*)

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developed a relatively simple gravimetric procedure for estimating the total weight of bacteria in faeces which they applied in their previous study (Stephen & Cummings, 1980*a*). This method represents an interesting possibility for studying the relationships between the amount, as well as the kind, of DF in the diet and the amount of bacteria in faeces, thereby increasing our understanding of the mechanisms involved in the faecal bulking effect of DF.

It has not been possible, however, to explain the ability of DF of low digestibility to increase faecal output by increased excretion of faecal bacteria. For example, wheat bran which contains DF of comparatively low digestibility, has in many studies (Kelsay, 1978; Stephen & Cummings, 1980*a*) been shown shown to be an effective faecal bulking agent, although its ability to stimulate microbial growth in the large intestine seems to be limited (Stephen & Cummings, 1980*a*). Thus it was suggested (Stephen & Cummings, 1980*a*) that the fraction of DF in bran which is not broken down by the intestinal microflora is able to bind water in the colon, thereby increasing faecal weight. However, it has also been shown (Stephen & Cummings, 1979) that the water-holding capacity of bran is low in comparison with other kinds of DF. Thus, the mechanisms behind the faecal bulking effect of bran are not yet completely understood.

The stool-bulking property of DF has been suggested as part of the explanation for consumption of fibre-rich diets, being found to be associated with low frequencies of certain diseases (Burkitt & Trowell, 1975). Based on the evidence quoted previously, it appears that the mechanisms behind this property of DF may be related to digestibility. Thus, in the present study, weight and composition of faeces from subjects consuming diets containing DF with different digestibility, were noted. A low-fibre diet was also included in the study. Conventional foods rather than concentrated fibre sources were used. Oral intake and faecal excretion of DF were measured and its digestibility calculated. The contribution of bacteria to faecal bulk was estimated using the gravimetric method developed by Stephen & Cummings (1980*b*). By this method, faecal samples are separated into four fractions, each enriched with one of the following three components: undigested insoluble DF, bacteria, and soluble components. The water-holding capacity of one of these fractions, enriched in undigested insoluble DF, was studied to determine whether this capacity is likely to be a mechanism behind the faecal bulking effect of DF.

## MATERIALS AND METHODS

### *Experimental design*

Twenty-one apparently healthy young adults (Table 1), consumed four experimental diets during three balance experiments. All subjects consumed the experimental diets for 20 d, days 14–19 being the balance period when the faecal samples were collected for the present study.

### *Balance techniques*

The metabolic study was performed on an outpatient basis. Subjects came to the metabolic unit for breakfast, lunch and dinner throughout the experiments. No food or drinks other than those served with the meals were allowed. The purpose of the experiment was carefully explained to the subjects and the importance of strict adherence to the protocol was continuously stressed. The energy requirement of each subject was estimated before the experiment by keeping a 4 d record when all foods eaten were weighed or measured and their total energy content calculated using Swedish food tables (National Food Administration, 1978). During the experiment, all subjects were weighed daily before breakfast in light clothing. For all subjects, daily variation in body-weight was less than 0·5 kg above or below the average body-weight, during the 20 d of the experiment. For each

Table 1. *Sex, height and body-weight of subjects consuming the experimental diets*
(Mean values and standard deviations)

| Experimental diet | No. of subjects (males/females) | Body-wt (kg) | | Height (m) | | Comment |
|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | |
| A | 2/3 | 63 | 9 | 1·75 | 0·10 | Diet A in Expt 2* |
| $B_1$ | 2/3 | 64 | 8 | 1·73 | 0·08 | Diet B in Expt 1* |
| $B_2$ | 2/3 | 66 | 14 | 1·72 | 0·10 | Diet B in Expt 2* |
| C | 3/3 | 65 | 6 | 1·73 | 0·08 | Not previously reported |

* As reported by Göranzon & Forsum (1987).

experimental diet, daily menus containing the amount of energy needed by each subject, were formulated and served to that particular subject every day throughout the experiment. Subjects with high energy requirements were given more of all foods. Taking practical and culinary aspects into consideration, attempts were made to keep the proportions of different food items in daily menus for each experimental diet fairly constant. Consequently daily menus from each experimental diet contained the same DF sources in roughly the same proportions. All food served was always completely consumed. Thus each of the twenty-one subjects consumed the same amounts of energy and DF every day throughout the experiment. To estimate daily intakes of energy and DF of subjects accurately during the balance period, twenty-one duplicate portions were prepared. These contained the same amounts and kinds of foods, cooked in the same way, as the foods in the respective twenty-one daily menus, and were homogenized, lyophilized and analysed as described later. However, the amounts of DF-containing foods in daily menus from an experimental diet were sometimes very similar and thus only eighteen of the twenty-one duplicate portions were analysed for DF. Consequently the daily DF intake during the balance period of three subjects was calculated as the DF content of one appropriate duplicate portion, obtained by analysis, plus the DF content (calculated using values published by Theander & Westerlund, 1986) of the small amounts of DF-containing foods that differed between the analysed duplicate portion and the daily menu consumed by the subject. All faeces was quantitatively collected from day 4 of the experiment and stored at $-20°$. Each subject consumed 0·8 g carmine (Apoteksbolaget, Gothenburg) before breakfast on day 14 and after dinner on day 19. One pooled faecal collection corresponding to the appearance of carmine in the faeces was made for each subject and was considered to represent faecal production during 6 d. All faeces to be included in one pooled sample were thawed for 8–10 h at 4° and was then weighed and homogenized with a known quantity of water for 1–2 min using an Ultra-turrax homogenizer (Labassco, Stockholm). Portions of the homogenate were lyophilized. The effect of the homogenization procedure on the microscopic count of bacteria was checked in separate experiments (see p. 176).

### *Diets*

*General.* Chemical composition of individual menus, contribution of different DF sources to the total intake of DF and chemical composition of DF have been described previously for diets A, $B_1$ and $B_2$ (Göranzon & Forsum, 1987). The contents of resistant starch and DF, as well as the chemical composition of DF in all the experimental diets used in the present study, are given in Table 2.

*Diet A.* This diet was formulated to supply relatively ample amounts of DF of low digestibility. By calculation from food tables (Southgate *et al.* 1976), this diet was found to

Table 2. *Contents of dietary fibre (DF) and resistant starch in daily menus of experimental diets and the composition of DF in terms of neutral sugars, uronic acid and lignin*

(Mean values and standard deviations for five determinations, except diet C where there were six determinations)

| | Diet A | | Diet $B_1$ | | Diet $B_2$ | | Diet C | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Dietary fibre (g/d) | 57·6 | 13·5 | 71·4 | 1·7 | 55·0 | 6·0 | 12·0 | 4·1 |
| Resistant starch (g/d) | 2·1 | 0·7 | 11·4 | 2·7 | 9·8 | 1·8 | 0·8 | 0·2 |
| Composition of DF (g/kg DF) | | | | | | | | |
| Rhamnose | 7 | 2 | 17 | 2 | 16 | 2 | 7 | 1 |
| Arabinose | 156 | 12 | 141 | 12 | 134 | 1 | 174 | 45 |
| Xylose | 253 | 31 | 65 | 7 | 94 | 15 | 174 | 27 |
| Mannose | 26 | 0 | 19 | 0 | 25 | 4 | 25 | 4 |
| Galactose | 38 | 8 | 39 | 0 | 49 | 3 | 123 | 27 |
| Glucose | 383 | 37 | 584 | 14 | 509 | 24 | 113 | 27 |
| Uronic acid | 63 | 6 | 103 | 11 | 116 | 6 | 94 | 24 |
| Lignin | 75 | 6 | 34 | 2 | 57 | 4 | 283 | 85 |

supply 52–78 g DF/d, about 89 % being derived from cereals. Subjects consuming this diet received the following foods: breakfast whole-wheat bread, cheese, breakfast cereal, orange juice; lunch whole-wheat bread, herring, egg, cheese, raw carrots and cabbage; dinner unpolished rice, ham, green peas, crisp bread (made from whole-rye flour) and rose-hip cream. Butter and milk were served at all meals. On average, this diet supplied daily: 82 g fat, 104 g protein, 10530 kJ energy and 301 g starch, mono- and disaccharides (Göranzon & Forsum, 1987).

*Diets $B_1$ and $B_2$.* These diets were formulated to supply relatively ample amounts of DF of high digestibility. By calculation from food tables (Southgate *et al.* 1976), diets $B_1$ and $B_2$ were found to supply 56–61 and 52–63 g DF/d respectively, about 85 % being derived from pulses, vegetables, potatoes and fruit. Subjects consuming these diets received the following foods: breakfast white wheat bread, butter, cheese, cornflakes, raisins and orange; lunch white beans, ground beef, green peas, maize and red pepper; dinner potatoes, ham, cheese, green peas, white wheat bread and canned pears. Milk was served with all meals. The two diets were formulated to contain similar amounts and proportions of DF from these different foods. However, as shown in Table 2, chemical analysis showed that their contents as well as the composition of their DF were different. On average, diet $B_1$ supplied daily: 50 g fat, 110 g protein, 9 030 kJ energy and 270 g starch, mono- and disaccharides. The corresponding values for diet $B_2$ were 68 g fat, 105 g protein, 9990 kJ energy and 290 g starch, mono- and disaccharides (Göranzon & Forsum, 1987).

*Diet C.* This diet was formulated to be a low-fibre diet. The chemical composition of the individual menus is shown in Table 3. The subjects consuming this diet received the following foods: brakfast sour milk, cheese, orange juice and apple sauce; lunch macaroni, meat sauce (Köttfärsås; Indra, Helsingborg), milk and cheese; dinner fish fingers (Fisk med mandel; Findus, Bjuv), potatoes and a chocolate bar. White wheat bread, margarine and sugar were served at all meals.

### *Food analysis*

The duplicate portions were analysed for the following variables: protein (nitrogen × 6·25) (Official Swedish Agricultural Methods of Analyses, 1966); fat (Amtsblatt der Europäischen Gemeinschaften 1971); starch (Åman & Hesselman 1984), and glucose, lactose,

Table 3. *Contents of fat, protein and carbohydrates (g/d), and energy (kJ/d) in daily menus from diet C*

(Mean values and standard deviations for six determinations)

| | Mean | SD |
|---|---|---|
| Fat | 78·3 | 16·3 |
| Protein | 92·4 | 14·6 |
| Starch* | 168·0 | 48·0 |
| Lactose | 27·6 | 5·0 |
| Glucose | 8·7 | 1·6 |
| Saccharose | 46·0 | 7·1 |
| Fructose | 10·7 | 1·8 |
| Uronic acid | 1·1 | 0·3 |
| Lignin | 3·4 | 1·0 |
| Neutral sugars | 7·6 | 3·8 |
| Total solids | 443·8 | 85·0 |
| Energy† | 8680 | 1690 |

* Including resistant starch.

† Obtained by application of Atwater general factors 17, 37, 16 kJ/g protein, fat and carbohydrate respectively. In these calculations carbohydrates were estimated by difference, i.e. carbohydrate = total solids − protein − fat − ash.

fructose and sucrose (Henninger, 1979). Ash was determined after treating the samples at 600° for 12 h and total solids after drying to constant weight at 105°. Resistant starch was analysed as described by Englyst *et al.* (1982). Estimates of dietary fibre were obtained from analysis of neutral sugars, uronic acid and lignin (Theander & Westerlund, 1986) and expressed in polysaccharide units.

### *Faecal analysis*

Faecal weight was the weight of the pooled collection obtained as described previously (see p. 173). Faecal water was calculated from faecal weight, allowing for the amount of water added during homogenization, the loss of weight during freeze-drying and the amount of moisture in lyophilized faeces estimated after drying to constant weight at 105°. Faecal solids were calculated as faecal weight minus faecal water. Neutral-detergent fibre (NDF) was determined according to Van Soest & Wine (1967).

*Fractionation of faeces.* The lyophilized faecal samples were fractionated using a modification of the method described by Stephen & Cummings (1980*b*). The procedure involved the following steps: 0·5 g samples (duplicates) were stomached (Stomacher 80, Seward, St Edmunds) for 5 min with 30 ml formyl saline (formalin–(9 g sodium chloride/l), 1:99 v/v) containing sodium lauryl sulphate (1 g/l; SLS). The mixture was then filtered through fine nylon mesh (aperture size approximately 150 $\mu$m). The residue on the nylon mesh was treated in the same way three times more and then suspended in 10 ml formyl saline with SLS. The bacterial count was taken and the suspension then dialysed against distilled water for 72 h and dried to constant weight at 105°. This was fraction 1. The combined filtrate and washings contained dense particles which settled out rapidly on standing. These were separated from the fluid by leaving them to sediment for 5 min and then aspirating the supernatant fraction. The sediment was washed several times in formyl saline with SLS and the washings added to the filtrate. This sediment was fraction 2 and it underwent the same sampling, dialysis and drying procedure as fraction 1. The volume of the remaining solution was noted before it was thoroughly mixed, a sample taken for

bacterial count, and then centrifuged at 30000 *g* for 30 min. The supernatant fraction was discarded and precipitates from each tube were combined and centrifuged again. The final precipitate, fraction 3, was dialysed as described previously and dried to constant weight at 105°.

To estimate faecal soluble components (fraction 4), 0·5 g lyophilized faeces (duplicates) were stomached with 30 ml formyl saline without SLS four times as described previously. The filtrate and residue were recombined, centrifuged at 30000 *g* for 30 min and the supernatant fraction added to the filtrate. The pellet was dialysed against distilled water and dried to constant weight at 105°. The weight difference between this and the original sample was equal to the weight of fraction 4. The volume of the combined filtrate and supernatant fraction was measured, ethanol was added to obtain a solution containing 80% ethanol (ethanol–water, 80:20 v/v) and the precipitate recovered by centrifugation at 30000 *g* for 30 min and dried to constant weight at 105°. This sub-fraction is the ethanol-precipitable components in fraction 4 (EPC-F4).

*Microscopic counting of faecal bacteria.* The number of bacteria in fractions 1, 2 and 3 was counted under a fluorescence microscope fitted with Ploem optics (Leitz, Wetzlar, West Germany) after staining with acridine orange as described by Kronvall & Myhre (1977). Samples (20 $\mu$l) were placed on a microscope slide, the liquid evaporated with a hairdryer and the diameter of the evaporated sample measured. The sample was stained and the number of bacteria noted. An occular lens fitted with a graticule net was used for this purpose. Three to six counts were taken for each sample. The average coefficient of variation (%) of the microscopic count was 71–148 for fraction 1, 37–50 for fraction 2 and 24–34 for fraction 3. The total number of bacteria in faeces was calculated as the sum of the counts in fractions 1, 2 and 3. The average coefficient of variation of this estimate varied between 11 and 31% for the different diets. The effect of the procedure used to homogenize faeces on the microscopic count of bacteria was checked in the following way: a faecal sample was homogenized by gentle stirring with a spoon and ten samples were taken for counting. The homogenate was then subjected to treatment with the Ultra-turrax homogenizer, as described earlier, and another ten samples taken. No difference in the microscopic count of samples taken before or after treatment with the Ultra-turrax was found. A similar test was performed to determine whether the addition of water to faeces during homogenization influenced the faecal microscopic count. There was no difference between faecal samples homogenized with and without water.

*Water-holding capacity.* The water-holding capacity of fraction 1 from faeces of subjects consuming diets A, $B_1$, $B_2$, as well as that of bran (Kungsörnen, Stockholm) and pectin (Sigma, Stockholm) was estimated using the method described by Stephen & Cummings (1979). Fraction 1 from diet C was not investigated since the amount of material recovered during fractionation of faeces from subjects consuming this diet was too small. The material under study was placed in a sac of dialysis tubing and dialysed against artificial intestinal fluid. The sacs were weighed after 24 and 48 h and after a further 24 h of dialysis against the same fluid containing polyethylenegycol (PEG). In this way the osmotic pull of the colonic absorptive surface is simulated (Stephen & Cummings, 1979). All estimates were made in triplicate.

### *Statistical analysis*

Linear regression was performed as described by Armitage (1971). One-way analysis of variance with 20 df, followed by the Studentized range test for separation of means were used to test for significant differences between groups (Armitage, 1971). If the variances differed significantly between groups, attempts were made to transform the data to

Table 4. *Percentage of faecal solids recovered in fractions 1, 2, 3 and 4*, total recovery of faecal solids and percentage of the total number of bacteria in fractions 1, 2 and 3*

(Mean values and standard deviations for five determinations except diet C where there were six determinations)

| Fraction no.... | 1 | | 2 | | 3 | | 4 | | 1+2+3+4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet† | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| A | | | | | | | | | | |
| Percentage of faecal solids | 32·2 | 6·0 | 7·4 | 5·4 | 30·7 | 9·2 | 35·2 | 8·0 | 105·5 | 3·8 |
| Percentage of total bacteria | 0·2 | 0·3 | 3·6 | 3·9 | 96·4 | 3·5 | | | | |
| $B_1$ | | | | | | | | | | |
| Percentage of faecal solids | 11·2 | 5·4 | 22·1 | 5·9 | 35·6 | 12·1 | 32·4 | 2·9 | 101·3 | 1·7 |
| Percentage of total bacteria | 1·3 | 0·9 | 2·5 | 1·6 | 96·1 | 2·4 | | | | |
| $B_2$ | | | | | | | | | | |
| Percentage of faecal solids | 6·6 | 1·1 | 13·2 | 5·3 | 45·1 | 6·6 | 34·9 | 2·7 | 99·9 | 4·7 |
| Percentage of total bacteria | 0·2 | 0·2 | 1·9 | 1·2 | 97·8 | 1·2 | | | | |
| C | | | | | | | | | | |
| Percentage of faecal solids | 3·5 | 2·8 | 11·6 | 11·5 | 56·3 | 6·7 | 33·6 | 4·8 | 104·4 | 9·8 |
| Percentage of total bacteria | 0·2 | 0·2 | 0·8 | 0·7 | 99·0 | 0·8 | | | | |

* Fractions of faecal samples prepared by the gravimetric method developed by Stephen & Cummings (1980*b*); for details, see pp. 175–176.

† For details of diets, see Tables 2 and 3 and pp. 173–174.

eliminate this difference. If this was not possible, means were compared by the Mann-Whitney test (Armitage, 1971) or by Student's *t* test for unpaired observations (Armitage, 1971), if required with an appropriate reduction in df (approximate *t* test).

### *Ethical considerations*

The participation of human subjects in the present experiment was approved by the ethical committee of the Medical Faculty, University of Uppsala.

## RESULTS

### *Fractionation of faeces*

The percentage of total solids recovered in fractions 1, 2, 3 and 4 are shown in Table 4 together with the distribution of faecal bacteria in fractions 1, 2 and 3. For diet A, 32·2% of faecal solids were recovered in fraction 1 which was higher than for any of the other diets. The recovery of total solids in fraction 2 for diet $B_1$ was high in comparison with the other diets. For all diets, the total recovery of faecal solids was about 100%, and about one-third was recovered in fraction 4. Also, on average, more than 96% of total faecal bacteria were present in fraction 3 for all diets, while the amount of bacteria in fractions 1 and 2 was comparatively small.

### *Digestibility of DF, and faecal weight and composition*

Table 5 shows the daily output of NDF in faeces of the subjects consuming the experimental diets in the balance experiment. The digestibility of DF in these diets is also shown, as are variables describing the amount and composition of the faeces collected. Table 5 shows that subjects consuming diet A excreted significantly more NDF in faeces than subjects consuming any of the other diets, and that subjects consuming diet C excreted significantly less NDF than subjects consuming diets $B_1$ or $B_2$. Subjects consuming diet $B_1$ excreted more NDF than subjects consuming diet $B_2$, although the difference was not

Table 5. *Neutral-detergent fibre in faeces, faecal weight, faecal solids, faecal water and faecal outputs of fractions 1+2, fraction 3, fraction 4 and ethanol-precipitable components of fraction 4 (EPC-F4)* of subjects consuming the experimental diets during the balance period, and the digestibility of the dietary fibre (DF) in these diets, faecal weight, faecal solids and faecal water/g DF consumed by the subjects*

(Mean values and standard deviations for five determinations except diet C where there were six determinations)

| Diet† ... | A | | $B_1$ | | $B_2$ | | C | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Neutral-detergent fibre: (g/d)‡ | 21·9 | 8·0 | 8·6 | 2·8 | 5·2 | 2·2 | 1·0 | 0·6 |
| Digestibility of DF§ | 0·62 | 0·11 | 0·88 | 0·04 | 0·90 | 0·05 | 0·91 | 0·04 |
| Faecal wt: g/d‖ | 288 | 138 | 179 | 42 | 108 | 48 | 74 | 23 |
| g/g DF¶ | 4·86 | 1·59 | 2·51 | 0·56 | 1·93 | 0·67 | 6·34 | 1·49 |
| Faecal solids: g/d** | 58·1 | 20·0 | 35·6 | 3·9 | 25·4 | 3·4 | 21·0 | 6·0 |
| g/g DF†† | 1·00 | 0·25 | 0·50 | 0·06 | 0·46 | 0·07 | 1·82 | 0·45 |
| Faecal water: g/d‡‡ | 230 | 120 | 144 | 41 | 83 | 47 | 53 | 17 |
| g/g DF§§ | 3·86 | 1·42 | 2·01 | 0·55 | 1·47 | 0·67 | 4·52 | 1·11 |
| Fraction 1+2 (g/d)‖‖ | 23·0 | 8·3 | 12·1 | 4·8 | 5·0 | 1·4 | 2·7 | 1·7 |
| Fraction 3 (g/d)¶¶ | 16·5 | 2·2 | 12·4 | 3·7 | 11·4 | 2·2 | 11·8 | 3·7 |
| Fraction 4 (g/d)*** | 21·4 | 11·2 | 11·5 | 1·9 | 8·8 | 1·0 | 7·3 | 2·9 |
| EPC-F4 (g/d)††† | 6·9 | 3·5 | 4·2 | 0·6 | 4·2 | 1·0 | 2·6 | 1·2 |

* Fractions of faecal samples prepared by the gravimetric method developed by Stephen & Cummings (1980*b*); for details see pp. 175–176.

† For details of diets, see Tables 2 and 3 and pp. 173–174.

‡ After transformation to natural logarithms, one-way analysis of variance showed: diet A > diet $B_1$ ($P < 0·05$), diet A > diet $B_2$, diet A > diet C, diet $B_1$ > diet C, diet $B_2$ > diet C ($P < 0·01$).

§ After transformation (values raised to the second power) one-way analysis of variance showed: diet A < diet $B_1$, diet A < diet $B_2$, diet A < diet C ($P < 0·05$).

‖ After transformation to natural logarithms, one-way analysis of variance showed: diet A > diet $B_2$, diet A > diet C, diet $B_1$ > diet C ($P < 0·01$), diets $B_1+B_2$ > diet C, diet A > diets $B_1+B_2$ ($P < 0·05$).

¶ After transformation to natural logarithms, one-way analysis of variance showed: diet A > diet $B_1$, diet A > diet $B_2$, diet $B_1$ < diet C, diet $B_2$ < diet C ($P < 0·01$).

** Diet A > diet C, diet $B_1$ > diet $B_2$ ($P < 0·01$) using Mann-Whitney test; diet A > diet $B_2$, diet A > diets $B_1+B_2$ ($P < 0·05$) and diet $B_1$ > diet C ($P < 0·001$) using Student's *t* test.

†† Diet A > diet $B_1$, diet A > diet $B_2$, diet C > diet $B_1$, diet C > diet $B_2$ ($P < 0·01$) using Mann-Whitney test; diet C > diet A ($P < 0·01$) using Student's *t* test.

‡‡ After transformation to natural logarithms, one-way analysis of variance showed: diet A > diet $B_2$, diet A > diet C, diet $B_1$ > diet C, diets $B_1+B_2$ > diet C ($P < 0·01$), diet A > diets $B_1+B_2$ ($P < 0·05$).

§§ After transformation to natural logarithms, one-way analysis of variance showed: diet A > diet $B_1$ ($P < 0·05$), diet A > diet $B_2$, diet C > diet $B_1$, diet C > diet $B_2$ ($P < 0·01$).

‖‖ After transformation to natural logarithms one-way analysis of variance showed: diet A > diet $B_2$, diet A > diet C, diet $B_1$ > diet C, diet A > diets $B_1+B_2$ ($P < 0·01$), diet $B_2$ > diet C, diet $B_1$ > diet $B_2$ ($P < 0·05$).

¶¶ One-way analysis of variance showed: diet A > diets $B_1+B_2$ ($P < 0·05$).

*** Diet A > diet $B_2$ ($P < 0·01$) and diet A > diets $B_1+B_2$ ($P < 0·05$) using Mann–Whitney test; diet $B_1$ > diet $B_2$, diet $B_1$ > diet C ($P < 0·05$) and diets $B_1+B_2$ > diet C ($P < 0·001$) according to Student's *t* test.

††† Diet A > diet C ($P < 0·05$) using Mann–Whitney test; diet $B_1$ > diet C and diet $B_2$ > diet C ($P < 0·05$) using Student's *t* test.

significant. The digestibility of the DF in diet A was significantly lower than that of the DF in any of the other diets. No significant difference in the digestibility of DF was found between diets $B_1$, $B_2$ and C.

The faecal weight of subjects consuming diet A was higher than that of subjects consuming the other diets, and subjects consuming diet C produced less faeces than subjects consuming diets $B_1$ or $B_2$. The differences between diets A and $B_2$, and between diets A and $B_1+B_2$ were significant. The differences between diet C on the one hand and diet $B_1$ or diets

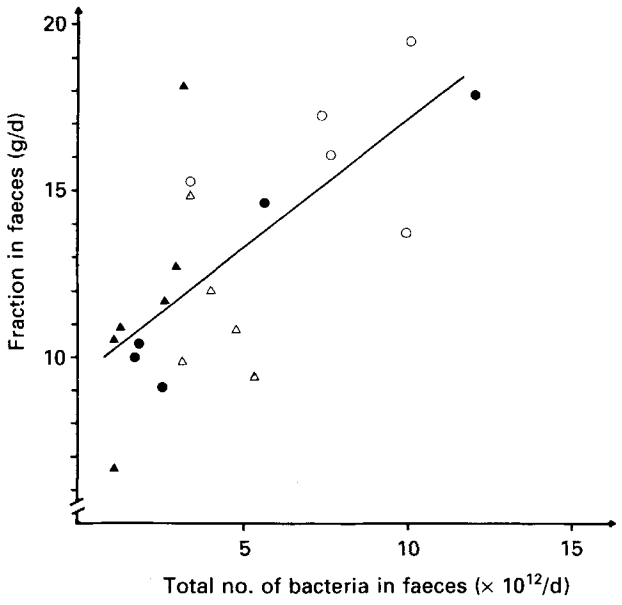


Fig. 1. Relationship between daily excretions of fraction 3 in faeces ($y$) and total number of bacteria in faeces ($x$) for subjects consuming the experimental diets during the balance period. Diet A (○), diet $B_1$ (●), diet $B_2$ (△) and diet C (▲). Regression equation: $y = 0{\cdot}758x + 9{\cdot}52$ ($r$ 0·69, $P < 0{\cdot}001$). Fraction 3 is obtained by fractionation of faecal samples by the gravimetric method of Stephen & Cummings (1980*b*). For details of diets, see Tables 2 and 3 and pp. 173–174.

$B_1 + B_2$ on the other hand were also significant. Faecal weight of subjects consuming diet $B_1$ was higher than that of subjects consuming diet $B_2$ but the difference was not significant. Similar observations were made with respect to the daily excretions of faecal solids and faecal water. Furthermore, Table 5 shows that if faecal weight, faecal solids and faecal water are expressed per g DF in the diet, the values obtained for diet A were significantly higher than those obtained for diet $B_1$ or diet $B_2$. Values for diet $B_1$ were higher than diet $B_2$ for these three variables, but the differences were in no case significant.

Table 5 also shows that daily excretions of fractions 1 + 2 were significantly higher for subjects consuming diet A than for subjects consuming diets $B_2$, $B_1 + B_2$ or C and that the corresponding value for subjects consuming diet C was significantly lower than that for subjects consuming diets $B_1$ or $B_2$. A significant linear relationship was found between faecal output (g/d) of fractions 1 + 2($y$) and NDF($x$):

$$y = 0{\cdot}994x + 1{\cdot}59 \ (r\ 0{\cdot}96,\ P < 0{\cdot}001)$$

as well as between faecal output (g/d) of fraction 1($y$) and NDF($x$)

$$y = 0{\cdot}807x - 1{\cdot}19 \ (r\ 0{\cdot}95,\ P < 0{\cdot}001).$$

Furthermore, Table 5 shows that the daily excretion of fraction 3 in faeces of the subjects was fairly similar regardless of the diet consumed. However, when compared with diets $B_1 + B_2$ the value obtained for diet A was significantly higher. Fig. 1 shows that the values obtained in the present study could be used to demonstrate a significant linear relationship between the size of fraction 3 and the total number of faecal bacteria. If instead the total number of bacteria in fraction 3 per day was used as the independent variable, the following regression equation was obtained:

$$y = 0{\cdot}769x + 9{\cdot}56 \ (r\ 0{\cdot}68,\ P < 0{\cdot}001).$$

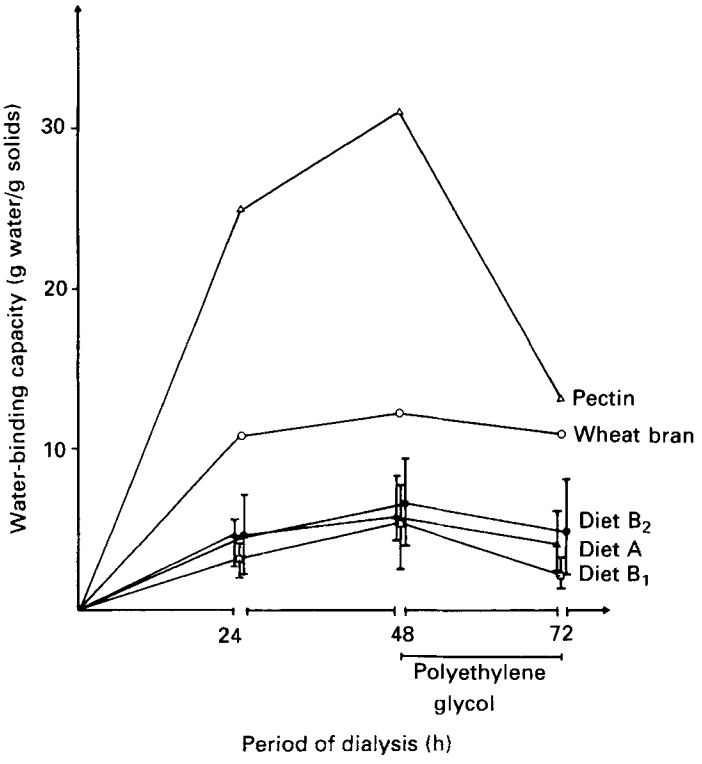


Fig. 2. Water-holding capacity (g water/g solids) after dialysis of fraction 1 from faeces of subjects consuming diets A (▲), $B_1$ (□) and $B_2$ (●) as well as by wheat bran (○) and pectin (△) against artificial intestinal fluid for 24 and 48 h as well as after a further 24 h against the same fluid containing polyethylene glycol. For the experimental diets, the values shown are mean values, and standard deviations represented by vertical bars, based on estimation of samples from five subjects. Fraction 1 is obtained by fractionation of faecal samples by the gravimetric method of Stephen & Cummings (1980*b*). For details of diets, see Tables 2 and 3 and pp. 173–174.

Subjects consuming diet A excreted more fraction 4 per day than did subjects consuming the other diets, as shown in Table 5. The differences between diet A and diets $B_2$ and $B_1+B_2$ were significant. The corresponding value for subjects consuming diet C was significantly lower than that for subjects consuming diets $B_1$ or $B_1+B_2$. A significant linear relationship was found between the output (g/d) of fraction 4 ($x$) and faecal water ($y$):

$$y = 10{\cdot}88x - 2{\cdot}99 \ (r\ 0{\cdot}92,\ P < 0{\cdot}001).$$

Finally, Table 5 shows that subjects consuming diet C excreted significantly less EPC-F4 daily in faeces than did subjects consuming any of the other three experimental diets. Similar values for this variable were obtained for diets $B_1$ and $B_2$, while the value for diet A was higher. The daily excretion of EPC-F4 by subjects consuming diet A was not significantly different from that of subjects consuming diets $B_1$, $B_2$ or $B_1+B_2$. Significant linear relationships were obtained between the output (g/d) of EPC-F4 ($x$) and faecal weight ($y$):

$$y = 42{\cdot}0x - 26{\cdot}2 \ (r\ 0{\cdot}92,\ P < 0{\cdot}001),$$

as well as with faecal water ($y$):

$$y = 35{\cdot}4x - 31{\cdot}7 \ (r\ 0{\cdot}91,\ P < 0{\cdot}001).$$

*Water-holding capacity of fraction 1*

Fig. 2 shows the water-holding capacity of fraction 1 from faeces of subjects consuming diets A, $B_1$ and $B_2$ compared with corresponding values obtained for wheat bran and pectin. The water-holding capacity of fraction 1 from subjects consuming diets A, $B_1$ and $B_2$ was lower than that of wheat bran after 24 and 48 h of dialysis against artificial intestinal fluid. Similar results were obtained after a further 24 h dialysis against the same fluid containing PEG. The water-holding capacity of bran was found to be lower than that of pectin on all occasions.

## DISCUSSION

According to the results shown in Table 5, we were successful in our attempts to formulate experimental fibre-rich diets, based on conventional foods, that contained DF with differing digestibilities. It should be noted, however, that we have for practical reasons, estimated DF in faeces and food by different methods, making it difficult to calculate the digestibility of DF in the experimental diets accurately. One important difference between the NDF method and the method described by Theander & Westerlund (1986) is that the latter, but not the former, includes soluble DF. However, it is possible to distinguish between soluble and non-soluble DF by the Theander & Westerlund (1986) method. Thus, we have also calculated the DF digestibility assuming that the DF intake equals insoluble DF estimated according to Theander & Westerlund (1986), while DF in faeces was assumed to equal NDF in faeces. These calculations showed the digestibility of DF in diets A, $B_1$ and $B_2$ to be 0·56, 0·87 and 0·89 respectively, supporting our conclusion that the digestibility of DF in diet A was lower than that of DF in diets $B_1$ and $B_2$. Also, subjects consuming the diet containing DF with low digestibility had higher faecal weights and excreted more water and solids in faeces than did subjects consuming similar quantities of DF of high digestibility. Thus our findings agree with the hypothesis that there is a relationship between the digestibility of DF and its ability to increase faecal bulk.

According to the results presented in Table 3, diet C provided about 3 g lignin/d. This was surprising since only foods with a low lignin content were included in this diet. However, the dishes served for lunch and dinner to subjects consuming this diet, were industrially prepared. It is known that during heat treatment, compounds may be formed by, for example, the Maillard reaction, that will be recovered as Klason lignin (Dreher, 1987). We assume that this is one possible explanation for the high lignin content of diet C.

Our results also indicate that diets $B_1$ and $B_2$ differed slightly with respect to their effect on faecal weight and composition. This is noteworthy because, although they were formulated to contain similar amounts and kinds of fibre-containing foods, it was found that the content and composition of the DF in these two diets were in fact different. The level of DF and the content of glucose in the DF were found to be higher for diet $B_1$ than for diet $B_2$ (Table 2). It is conceivable that such a difference could be due to different levels of resistant starch in the two diets. However, chemical analysis showed that diets $B_1$ and $B_2$ contained similar levels of this component (Table 2). The most likely explanation for our findings is the inherent variation in the amount and composition of DF in foods. Since diets $B_1$ and $B_2$ were found to differ in several respects, although the food they contained were very similar, the results obtained were treated both separately and combined.

A technical detail should be noted before discussing the results obtained by the fractionation procedure. Strictly following the description given by Stephen & Cummings (1980*b*) resulted in recoveries above 100% when the amount of total solids obtained in fractions 1–4 were added together. This problem was especially pronounced for diet A where fraction 1 was comparatively big, the consequence being that a substantial amount

of buffer solutes was recovered as total solids in this fraction unless removed by, for example, dialysis. Thus, fractionated samples were dialysed against several changes of distilled water before drying and weighing, and as shown in Table 4, recoveries of about 100% were obtained for all diets.

The total amount of bacteria per g faeces in the present study, as estimated by the microscopic count, was $10^{10}$–$10^{11}$. Values in the order of $10^{13}$ bacteria/g faeces have been obtained by sophisticated anaerobic techniques (Finegold *et al.* 1983). However, our values are similar to those for faecal bacterial count given by Cummings (1983). Stephen & Cummings (1980*b*) found a bacterial content of $10^{11}$/g faeces bsed on microscopic counts of bacteria after Gram staining in their study of faeces from subjects consuming a low-fibre British diet. In our study, samples were stained with acridine orange before counting. This dye has a differential staining effect at low pH with orange colouring of bacteria in contrast to green-to-yellow staining of human cells and background material (Kronvall & Myhre, 1977). Apparently, our slightly different technique gave values very similar to those obtained by Stephen & Cummings (1980*b*).

The method published by Stephen & Cummings (1980*b*) is supposed to fractionate faecal solids into undigested DF, faecal bacteria and faecal soluble components. The interpretation of our results in these terms is discussed below.

Stephen & Cummings (1980*b*) suggested that fraction 1 in their study contained undigested fibre, presumably of dietary origin. They reported that this fraction appeared, to the naked eye, to be mostly plant material. This is in agreement with observations made during the present study. When stained with acridine orange and viewed under the microscope it was obvious that the main part of this fraction was of non-bacterial origin.

The recovery of faecal solids in fraction 2 was higher in the present study than in the study reported by Stephen & Cummings (1980*b*). For example, in our study 11·6% of faecal solids were recovered in this fraction for diet C, while the corresponding value for the low-fibre diet studied by Stephen & Cummings (1980*b*) was only 2·2%. It may be possible that the procedure used to homogenize faeces in the present study tended to reduce undigested DF to comparatively small particles so that such faecal components were recovered in fraction 2 rather than in fraction 1. It is also possible that this difference is related to differences in the structure of the DF in the experimental diets used in the two studies. Under the microscope, fraction 2 looked very similar to fraction 1, except that the particles were smaller. The microscopic count also indicated that the bacterial content of this fraction was small. Thus it seems reasonable to regard the material in fraction 2, as well as in fraction 1, as being largely DF that had escaped digestion. The observation that daily excretion of NDF correlated with daily excretion of fraction 1, as well as with that of fractions 1 + 2, also support this conclusion.

Stephen & Cummings (1980*b*) fractionated faeces from subjects consuming a low-fibre British diet. That diet, as well as diet C in our study, both contained low levels of DF and comparatively high levels of fat and could thus be regarded as typical Western diets. It is of interest to note that fraction 3 from faeces of subjects consuming these two diets was similar, on a total solids basis, 14·1 *v.* 11·8 g/d. It is unlikely that such results would have been obtained if recovery of bacteria in fraction 3 had differed considerably between the two studies. This is noteworthy since the procedures used by us during processing and fractionation of faeces differed in some respects from the corresponding procedures used by Stephen & Cummings (1980*b*). These authors considered fraction 3 in their study to be 'a relatively complete and pure isolate of the bacteria from human faeces'. In our study, microscopic examination also indicated that fraction 3 contained mainly bacteria, with only minor contamination of other kinds of material. The microscopic count indicated that for all diets, the main part (>95%) of faecal bacteria was recovered in this fraction. We

therefore agree that it is likely that fraction 3 is a relatively complete and pure isolate of faecal bacteria. However, we consider it important to emphasize that it is not possible to present definite evidence for this statement. Nevertheless, the significant correlation obtained in the present study between the microscopic count of faecal bacteria and the size of fraction 3 seems to indicate that the latter could in fact be regarded as a relative, if not an absolute, indication of the amount of bacteria in human faeces.

The faecal samples collected in the present study apparently contained a substantial proportion of soluble material (Table 4), the consequence being that the daily output of fraction 4 was quite considerable for all subjects in the study (Table 5). We do not know the chemical constituents of this fraction but we consider it likely that, at least when collected from subjects consuming high-fibre diets, part of it consists of water-soluble undigested DF. Since the chemical constituents of DF, with the exception of lignin, are carbohydrates, soluble undigested DF should to some extent be precipitated in 80% ethanol. However, the solubility of organic compounds in 80% ethanol tends to decrease as their molecular weight increases and water-soluble undigested DF may also consist of low-molecular-weight carbohydrate compounds. Furthermore, it is conceivable that other kinds of material also insoluble in 80% ethanol, for example of endogenous origin, are present in faeces. Thus a high faecal excretion of EPC-F4 can only indicate, but not prove, to what extent undigested soluble DF is present in faeces.

One mechanism that has been suggested for the increase in stool output associated with consumption of DF is the ability of undigested DF to bind water in the large intestine. The water-holding capacity in vitro of the material recovered in fraction 1 was low when compared with bran. This capacity of bran was, as reported previously by Stephen & Cummings (1979), considerably lower than that of pectin which is generally considered to be DF with a comparatively high water-holding capacity. There are certainly difficulties in interpreting the results obtained by in vitro techniques for in vivo conditions, but low water-holding capacity of undigested, insoluble DF recovered from faeces is entirely possible. The water-holding capacity of DF is believed to be due to the three-dimensional structure of the polysaccharide chains and the presence of ionic groups in the molecules (Stephen & Cummings, 1979). It is conceivable that the hydrophilic parts of DF are attacked by the bacteria in the large intestine with the result that undigested insoluble DF will have lost its water-holding capacity when recovered in faeces.

Our findings could be examined in relation to mechanisms previously suggested as explanations for the ability of DF to increase faecal output. We found no support for the statement that increased amounts of bacteria in faeces is an important mechanism behind the ability of DF of high digestibility to increase faecal output. Our results may appear unexpected in view of the high excretion of faecal bacteria reported for subjects consuming cabbage fibre which has a high digestibility (Stephen & Cummings, 1980*a*). However, Cummings (1982) has also pointed out that consumption of DF with high digestibility does not necessarily result in big increases in stool output. For example, pectin appears to have no effect on faecal bulk (Durrington *et al.* 1976). These observations, together with the results presented in the present study, suggest that consumption of DF of high digestibility does not necessarily lead to a significant proliferation of the intestinal microflora.

Our results indicate that the excretion of faecal bacteria by subjects consuming diet A was slightly greater than that of subjects consuming the other diets. Drasar *et al.* (1976) reported similar findings for subjects consuming a diet containing bran. However, the difference in faecal output of fraction 3 between subjects consuming diets A and C in the present study could only explain a small part of the higher faecal weight of the former subjects as compared with the latter. Nor do our findings indicate that the water-holding capacity of insoluble undigested DF in faeces is a likely explanation for the finding that

Table 6. *Calculations showing faecal water associated with fractions 1+2, and with fraction 3 and faecal water not accounted for**

(Values used in these calculations are from Table 5)

| Diet†... | A | $B_1$ | $B_2$ |
|---|---|---|---|
| Faecal water associated with: | | | |
| Fractions 1+2 (g/d)‡ | 115 | 61 | 25 |
| Fraction 3 (g/d)§ | 39 | 29 | 27 |
| Faecal water not accounted for (g/d) ‖ | 76 | 54 | 31 |

* Fractions of faecal samples prepared by the gravimetric method developed by Stephen & Cummings (1980*b*), for details see pp. 175–176.

† For details of diets, see Tables 2 and 3 and pp. 173–174.

‡ Assuming a water-holding capacity of 5 g/g by fractions 1+2.

§ Assuming that fraction 3 consists of faecal bacteria and that faecal bacteria contain 70 g water/kg.

‖ Faecal water minus water associated with fractions 1+2 minus water associated with fraction 3.

subjects consuming diet A produced large amounts of faeces. In Table 6 values are given to illustrate that the water-holding capacity of material in fractions 1+2 is insufficient to account for all the faecal water of subjects on diets A, $B_1$ and $B_2$. The amount of water not accounted for seems to be related to faecal weight. Since faecal weight seems to be related to the kind of DF consumed, an important question is whether the kind of DF consumed is related to the amount of faecal water not accounted for.

Bearing in mind the limitations of the findings presented in Table 6, we would like to suggest that the following mechanism is the explanation for our observations. In the present study, a significant relationship between daily outputs of water and soluble components in faeces was observed. It may be of interest to consider this relationship since water is the main component in faeces. It is conceivable that, during fermentation of DF in the large intestine, small carbohydrate molecules are solubilized in the water phase of the intestinal contents. It is also conceivable that it is less likely that such a molecule will be used as a substrate by the microflora if it comes from a poorly fermentable DF, i.e. of low digestibility, than if it is derived from an easily fermentable DF, i.e. of high digestibility. If not fermented, the molecule will remain in the water phase and contribute to the osmotic properties of the fluid in the large intestine in a way that favours retention of water in the gut. It is also conceivable that such molecules could form gels in the large intestine, thereby contributing to faecal bulk. However, if the solubilized carbohydrate molecule is fermented, it will be converted into short-chain fatty acids that are absorbed and thus removed from the large intestine. This suggested model for the influence of DF on the amount of water in the large intestine has the merit that it could explain why DF of low digestibility are more effective faecal bulking agents than DF of high digestibility. The significant relationships obtained between EPC-F4 and faecal weight, as well as between EPC-F4 and faecal water, could also support this proposed model. It is important to emphasize, however, that more evidence is needed before it can be concluded that a mechanism of this kind is of importance in the faecal bulking properties of DF.

On the basis of our results, we would like to draw the following conclusions: our findings support the statement that the ability of DF to increase faecal output is related to its digestibility, while they do not indicate that consumption of DF of high digestibility is necessarily associated with increased excretion of faecal bacteria. Furthermore, non-soluble faecal material, likely to be of dietary origin and isolated from faeces of subjects consuming high-fibre diets, was found to have a low water-holding capacity. This finding indicates that mechanisms other than water holding of insoluble, undigested DF may play a part in the

ability of DF to increase faecal output. Finally, we propose that the amounts and properties of soluble compounds derived from DF are important in the faecal bulking effect. Our findings were obtained using diets where DF was derived from conventional foods only and is thus likely to be more representative of how fibre-rich conventional foods affect faecal weight and composition than results from studies based on fibre concentrates. However, further investigation is needed before we can fully understand to what extent and why such foods are able to increase faecal output and evaluate the significance of this property of DF to human health.

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