THE MICROBIAL CONTRIBUTION TO HUMAN FAECAL MASS

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PLATE II

THE NORMAL daily faecal output in Britain is 100–200 g per person, of which 25–50 g is solid matter (Wyman et al., 1978). It is commonly thought that a major component of human faeces is undigested plant material or fibre, and that when stool output increases on addition of fibre to the diet, the increase is due to water held in the colon by this material. On an average British diet however, only 3–5 g/day of dietary fibre remains in the stool (Cummings et al., 1979) and, on microscopic examination, what remains appears lignified and physically inert and is unlikely to account for the water content of normal faeces.

The high nitrogen content of faeces, 6% of the dry weight (Cummings 1978), suggests that the bacterial component of faecal material may be larger than previously thought. Previous estimates of the bacterial component of the wet faecal mass are 30–40% (van Houte and Gibbons, 1966; Moore and Holdeman, 1975). These estimates were based on direct microscopic counts which were then converted to a weight, assuming an average size for the bacteria. A more accurate method of assessing bacterial mass would be to separate the microbial fraction from the other faecal material and weigh it. We have therefore developed a method that fractionates faeces into three main components: the bacteria, undigested fibre, and soluble substances. The procedure has been developed from techniques used to isolate microbial matter from the rumen (Hoogenraad and Hird, 1970; Smith and McAllan 1974), with several steps altered or omitted to improve the separation of bacteria from fibrous debris and to ensure the purity of the bacterial fraction. By this method, a direct estimate of the microbial contribution to the weight of the stool has been obtained.

MATERIALS AND METHODS

Faecal material

The faecal material was from nine healthy male subjects aged 22–38 who were taking part in a metabolically controlled diet study (Cummings et al., 1978). In this study the subjects ate, for 3 weeks, a standard British-type diet containing 385 g of carbohydrate, 85 g of protein, 108 g of fat, 22 g of dietary fibre which provided 11·6 MJ/day. Faeces collected during the last 7 days of the

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diet were mixed, weighed and freeze-dried in an Edwards EF6 shelf freeze dryer for 1 week. Previous tests had shown that this was the longest time required to dry weekly collections of faeces. After a week, the faeces were removed and reweighed to give a value for total faecal solids. The dried material was then crushed with a rolling pin and thoroughly mixed, and portions were taken for the fractionation procedure, for measurement of faecal neutral detergent fibre (Goering and Van Soest, 1970), and for total nitrogen determination by the Kjeldahl method. Mean transit time through the gut was measured by the continuous marker method of Cummings, Jenkins and Wiggins (1976).

Fractionation procedure

The following procedure was carried out with duplicate samples of each freeze-dried 7-day faecal collection. Into an 80-ml Stomacher bag (Stomacher 80, Colworth), 0.5 g freeze-dried faeces were weighed accurately. To this was added 30 ml of formylsaline (NaCl 0.9% w/v and formalin 1% v/v) and 0.3 ml of 10% sodium lauryl sulphate (SLS). The bag and contents were thoroughly stomached for about 5 min. and the mixture was filtered through fine nylon mesh

\[
0.5 \, \text{g freeze-dried faeces} \quad \text{Stomach 5-10 min. with 30 ml formylsaline & 0.1\% SLS (a)} \quad \text{Filter through nylon mesh under vacuum (b)} \quad \text{Repeat (a) and (b) three more times}
\]

- RESIDUE
  - Centrifuge: 30 000 g for 30 min. (c)
  - Discard supernatant (d)
  - Mix with formylsaline and SLS
  - Remove 1 ml for counting
  - Repeat (c) and (d)
  - Dry over P₂O₅ and weigh
  - FRACTION A

- FILTRATE
  - Allow to stand for 5 min. then remove supernatant from sediment
  - SEDIMENT
    - Centrifuge: 30 000 g 30 min (e)
    - Discard supernatant (f)
    - Make up to 10 ml and take 1 ml for counting
    - Repeat (e) and (f)
    - Dry over P₂O₅
    - FRACTION B

- SUPERNATANT (≈800 ml)
  - Measure volume and mix thoroughly; take 5 ml for counting
  - Centrifuge: 30 000 g for 30 min.
  - PELLET
    - Weigh
    - SUPERNATANT
      - Sample for counting
      - FRACTION C

Dry over P₂O₅ (≈10 days)

Fig. 1.—Outline of technique for the fractionation of faeces into its main components. SLS = sodium lauryl sulphate
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(aperture size approximately 150 μm). Because the procedure produced considerable foaming, filtering was done under vacuum. The residue was then washed several times with formylsaline.

The residue on the nylon mesh was then washed into a second stomacher bag with another 30 ml of formylsaline, again with detergent, and the stomaching and filtering were repeated. This procedure was done four times in all and resulted in a residue of coarse particles retained on the nylon mesh and about 700 ml of combined filtrate and washings.

The fibrous residue was transferred into a weighed 50-ml centrifuge tube with formylsaline and spun at 30,000 g (MSE HiSpin 21) for 30 min. The supernatant was discarded and the tube weighed; 9.9 ml of formylsaline and 0.1 ml of SLS were added, the tube contents were thoroughly mixed in a whirlymix and a 1-ml sample was removed for microscopic counts of any bacteria present. The remainder was respun, the supernatant discarded, and the residue weighed and dried over P₂O₅. This was called fraction A.

The 700 ml of filtrate and washings from the separation of fraction A was noted to contain a small number of dense particles which settled out rapidly on standing. Microscopy suggested that they were simply smaller particles of fibrous debris that had passed through the nylon mesh. In ruminant work, such particles are removed by centrifuging at low speed, for example 200 g (Hoogenraad and Hird, 1970), but we found that this procedure also precipitated significant amounts of microbial matter. The particles were therefore removed from the fluid by leaving them to sediment for 5 min. and then aspirating the supernatant. The sediment was washed several times in a 10-ml tube to remove all traces of bacteria, and the washings were added to the 700 ml of filtrate. This sediment was called fraction B and underwent the same centrifugation and sampling procedure for microbial counts as fraction A.

The volume of the remaining suspension was noted before it was thoroughly mixed, and a 5-ml sample was taken for counting. The rest was then centrifuged at 30,000 g for 30 min. in 50-ml centrifuge tubes. The supernatant was discarded and the precipitates from each tube were combined and respun. The final precipitate, called fraction C, was weighed and then dried over P₂O₅ under vacuum to constant weight. Drying time was about 10 days for this fraction. The whole procedure is summarised in fig. 1.

Water-soluble components

The amount of water-soluble material in the freeze-dried faeces was measured by taking 0.5-g samples of faeces, stomaching for 5 min. with 30 ml of formylsaline, but without detergent, and filtering through nylon mesh. The residue was again stomached with three further lots of formylsaline and then filtrate and residue were recombined, centrifuged at 30,000 g for 30 min. and the supernatant removed. The pellet was dried and its weight subtracted from the original weight of material. Loss in weight was equal to the weight of the soluble component. This procedure was done in duplicate.

Examination of the faecal fractions

Light microscopy. Slide preparations for microscopy were made of fractions A, B and C. These were examined for bacteria by Preston and Morrell's (1962) modification of the Gram stain, and for plant material after staining with safranin and light green (Purvis, Collier and Walls, 1966).

Microscopic counts. To determine the proportion of the total faecal bacteria in each fraction, microscopic counts were made on the duplicate samples of fraction A, diluted 1 in 10, and fraction C before centrifugation, from six subjects. Bacteria were counted in a Helber counting chamber (Hawksley, Lancing) by phase-contrast microscopy at a magnification of 500. For each duplicate sample, three separate portions were taken, after thorough mixing, and the bacteria present in 30 squares were counted. Therefore for each 7-day collection of faeces, six counts were done. The coefficient of variation for the triplicate counts was 26.9% (for fraction C). However, the coefficient of variation between the average of these three counts for the duplicate samples was only 12.5%.
The variability in the counts was not due to observer error because when two people counted identical fields of 30 squares for six different samples the coefficient of variation was 3.3%.

Electron microscopy. Fraction C was also examined by scanning electron microscopy. The wet pellet was prepared by dehydration through graded alcohols, 50%–100%, 1 h in each. It was then substituted from alcohol to liquid carbon dioxide and dried in a Polaron Critical-point Drying Apparatus. Sections from each surface of the fractured pellet were fixed to stubs with silver and sputtered with 35 nm of gold, in a Polaron Specimen Coating Apparatus Type E5000. The specimens were then viewed on a Cambridge S4 Scanning Electron Microscope.

Chemical analysis. The total monosaccharide content and composition in fraction C was determined for eight subjects. The pellet was heated in a boiling water bath with 0.5~H₂SO₄. The sugars released by this procedure were estimated by gas-liquid chromatography as alditol acetates. The insoluble residue was treated with 72% H₂SO₄ at 4°C for 48 h, and the hexoses released were estimated by the anthrone method to give the value for cellulose. The nitrogen content of fractions A and C was determined by the Kjeldahl method.

RESULTS

Table I shows the means of daily faecal weight, transit time, faecal solids and excretion of neutral detergent fibre for the nine subjects whilst on the controlled diet. Faecal weight, mean transit time and excretion of faecal solids were all within the normal range for subjects of this age and sex, whilst the values for faecal neutral detergent fibre are of the same order as those for dietary-fibre excretion, measured by the Southgate’s (1969) method, for four subjects on a similar low-fibre diet (Cummings et al., 1979).

Table II shows the contribution of each of the four fractions to total faecal solids. On average these four components, fractions A–C and soluble fraction, accounted for 98% of the total solids. The coefficient of variation for duplicate samples of faeces for fraction A was 10.7% and for fraction C 2.2%, indicating good reproducibility of the method.

**Table I**

*Means of daily faecal weight, transit time through the gut, faecal solids and excretion of neutral detergent fibre in nine healthy male subjects on a controlled diet*

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Weight of faeces (g/day)</th>
<th>Mean transit time (h)</th>
<th>Weight of faecal solids (g/day)</th>
<th>Weight of neutral detergent fibre g/day</th>
<th>% Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>87</td>
<td>22.8</td>
<td>3.7</td>
<td>16.2</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>89</td>
<td>24.1</td>
<td>4.0</td>
<td>16.6</td>
</tr>
<tr>
<td>3</td>
<td>107</td>
<td>62</td>
<td>28.8</td>
<td>4.4</td>
<td>15.3</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>42</td>
<td>29.1</td>
<td>4.1</td>
<td>14.0</td>
</tr>
<tr>
<td>5</td>
<td>66</td>
<td>117</td>
<td>23.4</td>
<td>3.6</td>
<td>15.4</td>
</tr>
<tr>
<td>6</td>
<td>89</td>
<td>80</td>
<td>28.3</td>
<td>5.0</td>
<td>17.7</td>
</tr>
<tr>
<td>7</td>
<td>102</td>
<td>94</td>
<td>29.6</td>
<td>5.5</td>
<td>18.6</td>
</tr>
<tr>
<td>8</td>
<td>102</td>
<td>54</td>
<td>27.5</td>
<td>4.1</td>
<td>14.9</td>
</tr>
<tr>
<td>9</td>
<td>102</td>
<td>41</td>
<td>28.6</td>
<td>6.2</td>
<td>21.7</td>
</tr>
<tr>
<td>Means</td>
<td>92.8</td>
<td>74</td>
<td>26.9</td>
<td>4.5</td>
<td>16.7</td>
</tr>
</tbody>
</table>
TABLE II
The proportion of daily faecal solids in nine healthy male subjects that was either soluble, or present in fraction A, B or C

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Soluble</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.1</td>
<td>11.6</td>
<td>1.6</td>
<td>54.4</td>
<td>93.7</td>
</tr>
<tr>
<td>2</td>
<td>25.4</td>
<td>13.8</td>
<td>1.8</td>
<td>59.9</td>
<td>100.9</td>
</tr>
<tr>
<td>3</td>
<td>27.8</td>
<td>14.6</td>
<td>0.6</td>
<td>54.2</td>
<td>97.2</td>
</tr>
<tr>
<td>4</td>
<td>16.5</td>
<td>22.0</td>
<td>1.5</td>
<td>60.2</td>
<td>100.2</td>
</tr>
<tr>
<td>5</td>
<td>20.8</td>
<td>27.1</td>
<td>4.5</td>
<td>45.1</td>
<td>97.5</td>
</tr>
<tr>
<td>6</td>
<td>22.9</td>
<td>19.0</td>
<td>2.9</td>
<td>58.4</td>
<td>103.2</td>
</tr>
<tr>
<td>7</td>
<td>28.2</td>
<td>15.0</td>
<td>2.1</td>
<td>48.6</td>
<td>93.9</td>
</tr>
<tr>
<td>8</td>
<td>21.1</td>
<td>17.6</td>
<td>2.8</td>
<td>56.8</td>
<td>98.3</td>
</tr>
<tr>
<td>9</td>
<td>27.3</td>
<td>16.2</td>
<td>1.6</td>
<td>54.6</td>
<td>99.7</td>
</tr>
<tr>
<td>Mean</td>
<td>24.0</td>
<td>17.4</td>
<td>2.2</td>
<td>54.7</td>
<td>98.3</td>
</tr>
<tr>
<td>SEM</td>
<td>1.3</td>
<td>1.6</td>
<td>0.4</td>
<td>1.7</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Fraction A

Fraction A appeared to the naked eye to be mostly plant material. When stained with safranin and light green and viewed under the microscope (fig. 2) there was a preponderance of highly lignified plant components, such as xylem and phloem and the skeletons of some whole cells.

Microscopic counts of fraction A showed that it contained only 3–4% of the total bacteria (table III). For subjects 1, 2, 3, and 6 fraction A was less than 2% of the total count, but for subjects 4 and 5, it was 3.8 and 8.5% respectively. This was because the separation of bacteria in the initial part of the fractionation technique was more difficult for the specimens from subjects 4 and 5, and this is reflected in the larger bacterial count in fraction A and also (table II) in the much higher proportion of the total solids assigned to this fraction.

Total nitrogen content of the faecal solids and of fractions A and C is shown in table IV for six subjects. Faecal nitrogen averaged 1.45 g per day of which only 0.08 g (5.5%) was present in fraction A. This fraction was 1.9% nitrogen confirming that only a very small part of it could possibly be bacterial.

Fraction B

Table II shows that fraction B was only 2.2% of the total faecal solids. Light microscopy suggested that it was made up of small irregular fragments of plant material with few bacteria. The particles were of similar size to bacteria and proved to be very difficult to separate from them during counting. Because it represented such a small proportion of the total solids, the possible microbial content of fraction B has been ignored.
TABLE III

Microscopic bacterial counts, per g of faecal solids of fractions A and C in six subjects on identical diets

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Bacterial count per g of faecal solids ± SEM in</th>
<th>Percentage of total count in fraction A (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fraction A $(10^9)$</td>
<td>fraction C $(10^{11})$</td>
</tr>
<tr>
<td>1</td>
<td>6.24 ± 0.94</td>
<td>5.42 ± 0.99</td>
</tr>
<tr>
<td>2</td>
<td>6.67 ± 1.17</td>
<td>3.11 ± 0.54</td>
</tr>
<tr>
<td>3</td>
<td>1.65 ± 0.27</td>
<td>4.63 ± 1.59</td>
</tr>
<tr>
<td>4</td>
<td>5.02 ± 0.48</td>
<td>3.72 ± 0.72</td>
</tr>
<tr>
<td>5</td>
<td>4.42 ± 0.82</td>
<td>3.16 ± 0.52</td>
</tr>
<tr>
<td>6</td>
<td>2.50 ± 0.59</td>
<td>4.39 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>17.06 ± 3.21</td>
<td>4.24 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>15.86 ± 7.66</td>
<td>4.25 ± 1.12</td>
</tr>
<tr>
<td></td>
<td>32.40 ± 12.01</td>
<td>3.18 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>30.27 ± 9.11</td>
<td>3.55 ± 1.66</td>
</tr>
<tr>
<td></td>
<td>9.57 ± 1.87</td>
<td>3.68 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>1.80 ± 0.31</td>
<td>3.80 ± 0.26</td>
</tr>
</tbody>
</table>

* Means of triplicate counts on each of two samples.
† Mean of all counts.

Fraction C

As shown in table II, fraction C was found to be the major component (mean 54.7%) of the faecal solids. Bacterial counts of this fraction gave a mean of $4.0 \times 10^{11}$/gram of dry faeces which, when compared to the counts in fraction A (table III), indicated that more than 95% of the total faecal bacteria were concentrated in fraction C. The supernatant from centrifuging this component contained less than 1% of the total counts and has been ignored.

TABLE IV

Total nitrogen excretion in faecal solids and in fractions A and C, for six subjects on identical diets

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Total nitrogen excretion (g/day) in faecal solids</th>
<th>Percentage of total nitrogen in A + C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fraction A</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>1.32</td>
<td>0.04 0.76</td>
</tr>
<tr>
<td>5</td>
<td>1.26</td>
<td>0.09 0.66</td>
</tr>
<tr>
<td>6</td>
<td>1.66</td>
<td>0.09 1.08</td>
</tr>
<tr>
<td>7</td>
<td>1.55</td>
<td>0.09 0.79</td>
</tr>
<tr>
<td>8</td>
<td>1.43</td>
<td>0.13 0.96</td>
</tr>
<tr>
<td>9</td>
<td>1.46</td>
<td>0.06 0.92</td>
</tr>
<tr>
<td>Means</td>
<td>1.45</td>
<td>0.08 0.86</td>
</tr>
</tbody>
</table>
FIG. 2.—Fraction A stained with safranin and light green. × 25.

FIG. 3.—Fraction C Gram stain. × 640.

FIG. 4.—Fraction C; scanning electron microscopy. × 5000.
Having established that virtually all the faecal microflora were concentrated in fraction C we examined its purity in several ways. Gram stains of several samples showed the pellet to be almost entirely bacterial with only occasional fragments of fibrous debris or human cells (fig. 3). Staining with safranin and light green also revealed some plant material, lignified structures being stained dark red or black, but the general picture was of a mass stained light green, as bacteria are represented by this stain.

Scanning electron microscopy also suggested that the pellet was largely bacterial. Both the upper and lower surfaces contained small amounts of non-microbial matter but when the pellet was broken into large pieces the fractured surfaces produced appeared to be entirely bacterial (fig. 4).

Fraction C was approximately 6% nitrogen, which is at the lower end of the normal range for mixed microbial populations in the rumen (Milwid, Oliver and Topps, 1968; Mason and Palmer, 1971).

Analysis of the bacterial pellet for sugars is shown in table V. The main

**Table V**

*Sugar analysis on fraction C (% dry weight)*

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>hexose:</th>
<th>pentose:</th>
<th>cellulose</th>
<th>total sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rhamnose + fucose</td>
<td>mannose</td>
<td>galactose</td>
<td>glucose</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>0.2</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>0.2</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
<td>0.2</td>
<td>1.1</td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<td>0.3</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>0.2</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>0.3</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>1.4</td>
<td>0.2</td>
<td>1.3</td>
<td>3.2</td>
</tr>
<tr>
<td>9</td>
<td>1.3</td>
<td>0.4</td>
<td>1.7</td>
<td>6.8</td>
</tr>
</tbody>
</table>

object of this procedure was to show whether there was any contamination of the pellet by plant material. Arabinose and xylose, as well as cellulose, are unlikely to be of bacterial origin whereas ribose may well be. Hexose, on the other hand, could be from either bacteria or plant material (Dr H. S. Wiggins, personal communication). From the table it can be seen that the maximum contamination of the pellet with plant material, if all hexose is from fibre, would be 10%. A more realistic estimate is 6–7% because some of the hexose is likely to be from bacteria.

**Discussion**

Quantitation of the microbial population in human faeces may be approached in several ways. Direct microscopic counts have been used to
estimate bacterial numbers (van Houte and Gibbons, 1966; Finegold, Attebery and Sutter, 1974) but these are subject to sampling error (Rall et al., 1970) and may underestimate total numbers, because bacteria tend to stick together and form clumps. In addition, it is difficult to extrapolate from the count to a weight of bacteria because the microbial population of the faeces contains a great variety of species, the weights of many of which are unknown.

Similar difficulties arise when chemical methods are used to determine bacterial mass, such as the determination of diaminopimelic acid (DAP). The DAP content of different species varies widely (Synge 1953; Purser and Buechler, 1966) and assumptions must be made when the weight is being converted to a mixed population. RNA has also been used as a microbial marker but even when it is rapidly degraded, as in the rumen, enough contamination remains from the diet to affect results (Smith et al., 1978); mucosal cells may also contribute to contamination.

The composition of rumen microflora has been studied by isolating bacteria by filtering and centrifugation (Hoogenraad and Hird, 1970; Smith and McAllan, 1974). This approach was also applied early in this century to the quantitation of the bacteria in human faeces and gave values of around 30% for the bacterial component of faecal solids, (MacNeal, Latzer and Kerr, 1909). Such direct methods have the advantage that no assumptions are necessary for calculation of the weight of bacteria, but because there were no ultracentrifuges at that time, the bacterial component was probably underestimated due to incomplete recovery of material from the faecal suspension.

The problem with separation techniques is that complete removal of bacteria from other material is difficult. Rumen isolation procedures do not require the complete separation of the microbial population from plant material and hence bacteria firmly associated with plant fragments are left behind. The almost complete removal of bacteria from other faecal material was achieved in the present study by the initial stomaching and filtering procedures in the presence of detergent; these steps are regarded as the key to the success of this method. Detergent, which is not used in rumen procedures, improves separation considerably. The detergent concentration used was that added to bacterial suspensions being prepared for direct microscopic counts (Meynell and Meynell, 1965) and is sufficient to reduce surface tension, without rupture of bacterial cells, as occurs with stronger concentrations of anionic detergents (Shafa and Salton, 1960).

From table III, it can be seen that only 2% of the total bacterial population was left in association with the plant fraction, fraction A. After the vigorous separation procedure with detergent, microscopic counts were subject to less error than counts on whole stools. However, the relatively high coefficient of variation (26.9%) for counts on triplicate portions of the same sample suggest that some problems with accuracy remain. This variation was not due to observer error but more probably to difficulty in taking a representative sample of only 0.03 ml from such a large volume and keeping the volume adequately mixed while sampling. For the purposes of comparison between the various fractions in this study counts were probably valid, although as a basis for
extrapolating to total faecal bacterial numbers they should be used with caution.

Microscopic counts provided evidence that more than 95% of the faecal microflora had been concentrated in fraction C. It was then necessary to ensure that this bacterial fraction was not contaminated with other faecal components. Contamination of the bacterial suspension was minimised by removal of small dense particles which sedimented on standing. This fraction, B, which was only 2% of the total solids, appeared on microscopy as small fragments of fibre or crystals.

The purity of the bacterial pellet, fraction C, was examined by both the Gram and the plant stains which showed that only a very small amount of plant material remained. This was confirmed by scanning electron microscopy (fig. 4) which showed a densely packed bacterial mass. Chemical analysis of the sugar component of the pellet suggested that possibly 6–7% of the pellet might be plant material (table V).

No other major contaminant of the bacterial pellet was seen. Occasionally, a human cell could be seen by Gram stain, but most sloughed cells from the intestinal epithelium are probably digested by bacteria in the gut lumen and do not reach the faeces (Hoskins, 1978). Other material said to be present in faeces, such as muscle fibres, connective tissue and crystals (Stitt, Clough and Branham, 1948) were not present in fraction C nor were seen in significant amounts in any part of the stool.

The nitrogen content of fraction C (table IV) was 6%. This value is at the lower end of the range of reported nitrogen values for mixed rumen populations (Milwid et al., 1968; Mason and Palmer, 1971). About 40% of the faecal nitrogen in this study appears to be in the soluble fraction of the solids. This is surprisingly high although, using a similar method, Tremolieres et al. (1960) suggested 47% for soluble nitrogen. The freeze-drying process and the stomaching with formylsaline may have ruptured some of the bacterial cells, giving higher values for soluble nitrogen and lower values in the bacterial pellet.

One faecal component that has not been considered is fat. The normal stool contains about 3 g of fat per day (Cummings, 1978). Bacterial fat content has been found to be 10–15% for pure cultures (Luria, 1960) and 12–20% for mixed rumen populations (Czerkawski, 1976). If, as our study suggests, about 55% of faecal solids are bacteria, about 70% of the faecal fat can be accounted for by its presence in bacteria; the remaining 1 g is unaccounted for but may have been solubilised by the detergent. On the top surface of fraction C when seen by scanning electron microscopy, there appeared to be crystalline material which could be fatty acid crystals. Contamination of the pellet by fat is therefore possible but it seems unlikely that it represents a substantial proportion of it.

Fraction C therefore appears to be a relatively complete and pure isolate of the bacteria from human faeces and represents 55% of the total faecal solids in these subjects on a typical British diet.

The method itself is fairly time consuming, fractionation of 4–5 samples
taking a working day. Repeated washings are necessary to ensure a good separation of bacteria from other structural material in the stool and this necessitates repeated centrifugation. Drying over P₂O₅ is also lengthy but could be shortened by freeze drying.

The proportion of faecal solids in fraction A—17.4% of the total solids—was similar to the mean neutral detergent fibre value (16.7%) (table I). However, faeces from subjects 4 and 5 were difficult to fractionate and even increasing the length of stomaching time and the frequency of washing could not reduce the fraction A value for subject 5 to that of the neutral detergent fibre value. Other subjects have been studied on different diets and none has presented this problem. It may therefore be less common than is suggested by the results from these nine subjects. Subject 5 had a very long mean transit time (117 h) with a high percentage of faecal solids (35.4%). This may mean that the bacteria and other material were more desiccated in the stool of subject 5 and therefore associated more closely. The method may therefore be difficult in some subjects but with experience the material on the nylon mesh (fraction A) can be inspected, problems in separation detected early, and appropriate steps taken to improve it. The overall reproducibility of the method was good, particularly for the bacterial fraction.

The results obtained from this fractionation technique show that the faecal bacterial mass is larger than previously thought. If bacteria are 80% water, 55% of the dry weight becomes an even larger proportion of the wet stool, about 75%. Previous studies have suggested 30% (van Houte and Gibbons, 1966) or 40% (Moore and Holdeman, 1975) but these are based on conversions from microscopic counts. The mean value obtained for counts in the present study was 4.0 × 10¹¹ (range 2.8–6.0 × 10¹¹) per g of dry faeces, which is similar to those from other studies (Finegold et al., 1974; Finegold et al., 1975; Mastro-marino, Reddy and Wynder, 1978). On the basis of conversion factors used in the past, the bacterial mass as represented by the weight of fraction C in this study would comprise many more bacteria than are indicated by the microscopic count. This suggests that either the counts are universally low or that the conversion factors used are wrong. Recent studies of faeces have identified new species of bacteria with diameters ranging from 0.3 to 1.0 μm (Gossling and Moore, 1975). Bacteria at the smaller end of this scale would not be seen easily at a magnification of × 500.

The contribution of bacteria to faecal weight is therefore very considerable and approximately twice previous estimates. This finding is supported by current views of the composition of the human faeces and might explain changes in faecal weight and composition associated with varying dietary-fibre intakes. It is also possible that the colon contains a much larger mass of bacteria than hitherto thought, the metabolic consequences of which, for the host, have not been fully explored.

**Summary**

A method has been developed, based on techniques used for isolating
bacteria from the rumen, that enables human faeces to be fractionated into three major components. The method requires repeated, vigorous agitation of a suspension of faecal solids with detergent, the use of a stomacher, and high-speed centrifugation. By this means the faecal microflora are separated from faecal dietary-fibre residues. These two components, with water-soluble material in the stool, comprise $98.3 \pm 0.9\%$ of faecal solids. The purity of the microbial fraction was demonstrated by Gram and plant stains and by scanning electron microscopy. Microscopic counts of the bacteria in each fraction of the stool showed that the microbial fraction contained $95\%$ of the total bacteria. Chemical analysis of the component sugars indicated $6-7\%$ possible contamination by non-bacterial polysaccharides. The bacterial pellet was $6\%$ nitrogen, and accounted for $60\%$ of the total faecal nitrogen. When faeces from nine healthy subjects on a metabolically controlled British-type diet were studied, bacteria comprised $54.7 \pm 1.7\%$ of the total solids, fibre $16.7 \pm 0.8\%$ and soluble material $24.0 \pm 1.3\%$. Bacteria therefore represent a much larger proportion of the faecal mass than was previously thought.

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