

Short chain fatty acids in human large intestine, portal, hepatic and venous blood

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SUMMARY Evidence for the occurrence of microbial breakdown of carbohydrate in the human colon has been sought by measuring short chain fatty acid (SCFA) concentrations in the contents of all regions of the large intestine and in portal, hepatic and peripheral venous blood obtained at autopsy of sudden death victims within four hours of death. Total SCFA concentration (mmol/kg) was low in the terminal ileum at 13 ± 6 but high in all regions of the colon ranging from 131 ± 9 in the caecum to 80 ± 11 in the descending colon. The presence of branched chain fatty acids was also noted. A significant trend from high to low concentrations was found on passing distally from caecum to descending colon. pH also changed with region from 5.6 ± 0.2 in the caecum to 6.6 ± 0.1 in the descending colon. pH and SCFA concentrations were inversely related. Total SCFA ($\mu\text{mol/l}$) in blood was, portal 375 ± 70 , hepatic 148 ± 42 and peripheral 79 ± 22 . In all samples acetate was the principal anion but molar ratios of the three principal SCFA changed on going from colonic contents to portal blood to hepatic vein indicating greater uptake of butyrate by the colonic epithelium and propionate by the liver. These data indicate that substantial carbohydrate, and possibly protein, fermentation is occurring in the human large intestine, principally in the caecum and ascending colon and that the large bowel may have a greater role to play in digestion than has previously been ascribed to it.

There is now much evidence¹⁻⁵ that dietary polysaccharides, both starch and non-starch polysaccharides (dietary fibre), escape digestion in the human small intestine and are then broken down in the large bowel.⁶⁻⁸ This phase of digestion is carried out by anaerobic bacteria that reside in the caecum and colon and may parallel that occurring in the rumen and hind gut of other animal species.⁹⁻¹⁰ The microbial breakdown of carbohydrate is an anaerobic process, known as fermentation, that yields short chain fatty acids (SCFA) (acetic, propionic and butyric acids) as its main end products together with various gases (hydrogen, methane and carbon

dioxide) and energy, which the bacteria require for growth and the maintenance of cellular function.

The production of hydrogen and methane from fermentation in the human large intestine has been known for many years,^{6,11} as has the presence in faeces of end products of fermentation such as SCFA.¹² More recent studies have shown that bacteria which produce polysaccharide degrading enzymes occur in the large bowel of man.¹³ Many lines of evidence therefore support the idea that fermentation occurs in the colon but, because of the difficulties in obtaining access to the human large bowel during normal digestion, most of the evidence for fermentation has, of necessity, been indirect. We have therefore tried to establish the occurrence of fermentation in the caecum and other regions of the colon, and the fate of its principal end products by measuring SCFA in all regions of the small and large

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intestine and in portal, hepatic and peripheral venous blood obtained soon after death from people who had died suddenly.

Methods

STUDIES IN PIGS

In order to determine the magnitude of post mortem change that might occur in the composition of gut contents five mature pigs, which had been allowed free access to food until the time of death, were slaughtered and pH was measured in the ileum, caecum, midcolon and rectal area at the time of death and four hours after death, during this period the bodies lay on the floor of the animal mortuary. From two pigs samples (about 5 g) were taken from each region at half hourly intervals from 0–4 h for SCFA analysis. In addition, to assess the variability in SCFA concentrations due to sampling six samples were taken from the caecum of one pig at one hour after death and duplicate samples from all other regions at the same time.

STUDIES IN MAN

Samples of blood and intestinal contents were obtained at the time of autopsy carried out a maximum of four hours after death. The abdomen was opened first and blood obtained from the portal vein, hepatic vein and then from a peripheral vein – usually the femoral. Blood was put into lithium-heparin containing tubes and plasma removed by centrifugation and then stored at -20°C before analysis. String was tied round the colon to delineate the anatomical regions (caecum, ascending, transverse, descending, sigmoid, rectum as defined in *Gray's anatomy*¹⁴) and then the whole of the intestine removed. The small intestine was divided into 100 cm lengths, after removal from the mesentery, and data described in the paper from the jejunum are from the first 100 cm distal to the duodenojejunal flexure, whilst ileal samples were obtained from the segment proximal to the ileocaecal valve. The colon was opened along its entire length and the contents scraped out with a wooden spatula, mixed, and placed in a plastic pot. pH was measured immediately using an EIL soil electrode, the mean of three separate measurements being taken in each region. All samples were then frozen in solid CO_2 and stored at -20°C until analysed.

CHEMICAL METHODS

Short chain fatty acids were measured in intestinal contents after acidification and extraction into diethyl-ether by gas liquid chromatography on a 1.4 m glass column packed with 10% FFAP on 100–120 mesh Chromosorb W-AN DMCS. This

method does not permit separation of the branched chain fatty acids 2-methyl-butyrate and isovalerate. Dicarboxylic acids were determined as methyl esters using the same column as described by Holdeman *et al.*¹⁵ Short chain fatty acids in blood were measured by gas liquid chromatography after acidification and freeze-transfer as described by Pomare *et al.*¹⁶ For portal and hepatic blood 2-methyl-butyrate was used as internal standard.

Statistical analysis was by analysis of variance and regression analysis with the SPP package for micro computers.

Results

PIG STUDIES

Figure 1 shows pH (in five pigs) for various regions of the intestine at the time of death and at four hours post mortem. No significant change in pH was observed between 0 and 4 h in any region (caecum 0 h pH 6.02, 4 h 6.12, t 1.2; mid-colon 0 h 5.89, 4 h 5.98, t 2.1; rectum 0 h 6.40, 4 h 6.29, t 1.4 ($n=5$ in all cases) but significant differences between regions were found ($F=30.06$, $p<0.001$).

For short chain fatty acid concentrations, which were measured at half-hourly intervals in two pigs only, no consistent trend with time was seen in any region (Fig. 2); ileum, coefficient of linear regression $b=0.20\pm 0.48$ (standard error) correlation coefficient $r=0.10$; caecum $b=3.37\pm 2.25$, $r=0.35$; mid-colon $b=-3.24\pm 3.54$, $r=0.22$; rectum $b=-4.90\pm 2.45$, $r=0.45$ ($n=18$ in all cases). When values for the caecum were recalculated for the first three hours only then a small, but significant, rise in SCFA was noted (b 5.35 ± 2.1 , $p<0.02$, $r=0.55$, $n=14$). Mean total SCFA concentrations (mmol/kg) were, ileum 15.6, caecum 83.0, mid colon 113.6 and rectum 70.4, and these differences were highly significant ($F=158.9$, $p<0.001$). Acetate was the predominant anion with molar ratios of acetate: propionate:

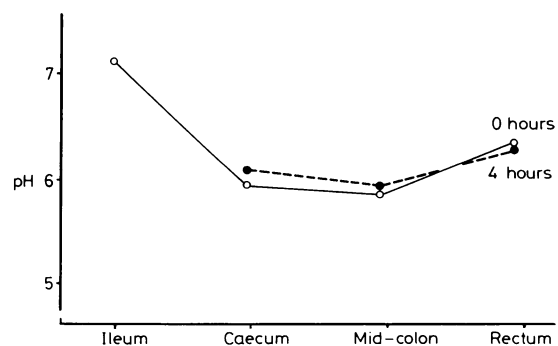


Fig. 1 pH of intestinal contents at time of death, and four hours after death in five mature pigs.

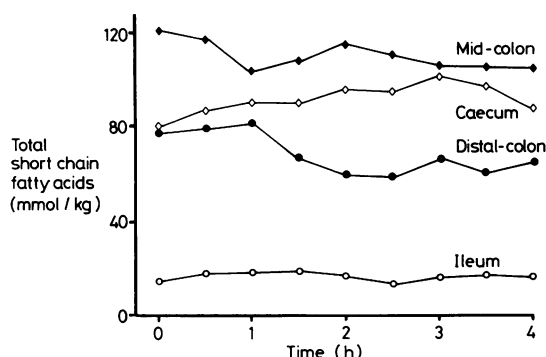


Fig. 2 Total short chain fatty acid concentrations (mmol/kg) in intestinal contents at the time of death and at 30 minute intervals for the ensuing four hours in two pigs.

butyrate of 97:0:3 in the ileum and 65:27:8 for all samples from the large intestine. Variation caused by sampling was moderate, ranging from 3.6–5.6% (coefficient of variation) for the three anions measured in six samples taken simultaneously from the caecum and was 3.6% for total SCFA measured in paired samples at 1 h from all four regions of the intestine ($n=28$). Mean variation with time, for all samples over 4 h in two pigs, ranged from 7.5% mid-colon to 13.2% rectum ($n=14$) and between pigs for total SCFA in all samples 6.4% ($n=56$).

HUMAN STUDIES

Details of the cases are given in Table 1. There were six subjects, two women, four men, average age 57 years, three of whom died suddenly from coronary heart disease and three were violent deaths. Most died between the hours of 9 am and midday and autopsy was done on average 3 h 20 min after death (range 2 h 15 min to 4 h 5 min). Small bowel contents weighed 291 g (range 156–508) and those in the large bowel 174 g (range 83–421). Large bowel contents varied in appearance from an amorphous pasty brown faecal material in the left colon to a more heterogenous mixture on the right side in which there were recognisable items of food such as tomato skin, sweetcorn, macerated vegetables, flakes of bran,

seeds, pips, etc. Forty six per cent of contents were in the caecum and ascending colon. Three subjects showed diverticular disease of the sigmoid colon and in one the appendix had been removed. Three colons were grossly normal (case 1, 4, 5, Table 1).

pH and total SCFA concentrations are shown in Figures 3 and 4. Total SCFA concentrations were low in the jejunum at less than 1 mmol/kg rising to 13 ± 6 (SEM) in the ileum but then by over 10-fold to 131 ± 9 in the caecum. Concentrations then fell progressively, 123 ± 12 (ascending), 117 ± 9 (transverse), 80 ± 17 (descending), and 100 ± 30 (sigmoid rectum). No difference in SCFA concentrations amongst the five regions of the colon was found by analysis of variance ($F 1.70$, $p=0.19$) but a significant trend between concentration and region was present ($b -15.2 \pm 4.2$, $p<0.002$; $r -0.60$, $p<0.01$, $n=25$) with concentration being higher in the right colon (Fig. 4). Similarly pH was significantly associated with region ($b 0.22 \pm 0.06$, $p<0.001$, $r 0.62$, $p<0.001$) being lower in the right colon (Fig. 3). pH and SCFA were significantly related ($b -0.22 \pm 0.06$, $p<0.002$;

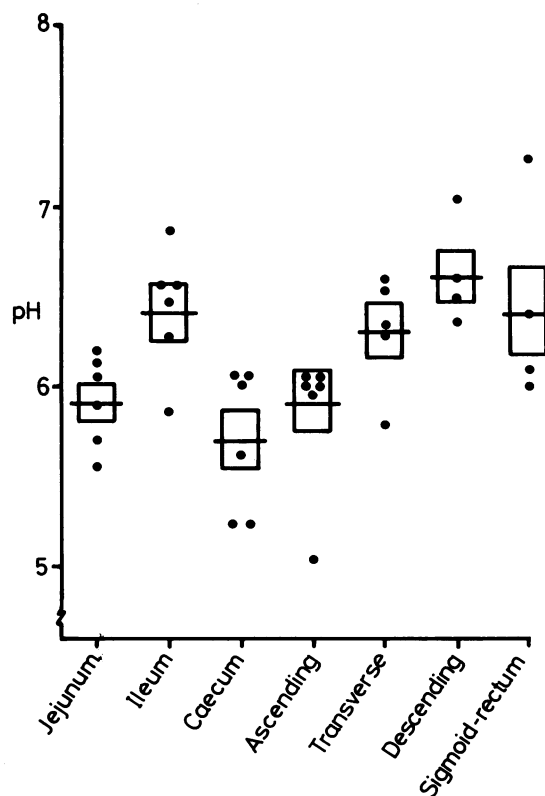


Fig. 3 pH of intestinal contents at between two and four hours after death in six people who had died suddenly. Box=Mean+1 SEM.

Table 1 Details of cases

| Case no | Sex | Age (year) | Time of death | Time of autopsy | Cause of death |
|---------|-----|------------|---------------|-----------------|------------------------|
| 1 | F | 16 | 1230 | 1445 | Road traffic accident |
| 2 | M | 66 | 0945 | 1300 | Coronary heart disease |
| 3 | M | 75 | 0920 | 1215 | Coronary heart disease |
| 4 | F | 62 | 1200 | 1545 | Road traffic accident |
| 5 | M | 36 | 1155 | 1600 | Shotgun injury |
| 6 | M | 89 | 0500 | 0830 | Coronary heart disease |
| Mean | | 57 | 1005 | 1320 | |

Table 2 pH, Short chain fatty acid and other organic anions* in the human intestine

| | Small intestine | | | Large Intestine | | | |
|--------------|-----------------|------------|------------|-----------------|------------|-------------|----------------|
| | Jejunum | Ileum | Caecum | Ascending | Transverse | Descending | Sigmoid/rectum |
| pH | 5.9 (0.1) | 6.3 (0.1) | 5.6 (0.2) | 5.7 (0.2) | 6.2 (0.1) | 6.6 (0.1) | 6.3 (0.2) |
| Acetate | 0.6 (0.6) | 7.9 (4.1) | 69.1 (5.0) | 63.4 (6.8) | 57.9 (5.4) | 43.5 (11.1) | 50.1 (16.2) |
| Propionate | — | 1.5 (1.0) | 25.3 (3.7) | 26.7 (4.0) | 23.1 (2.8) | 14.2 (3.1) | 19.5 (6.7) |
| Iso-butyrate | — | 0.3 (0.2) | 2.1 (0.4) | 1.8 (0.2) | 2.6 (0.5) | 2.3 (0.4) | 1.9 (0.8) |
| Butyrate | — | 2.3 (1.3) | 26.1 (3.8) | 24.5 (4.2) | 24.4 (2.2) | 14.7 (2.9) | 17.9 (5.6) |
| Iso-valerate | — | 0.1 (0.1) | 2.7 (0.5) | 2.7 (0.5) | 3.4 (0.4) | 3.5 (0.1) | 3.7 (0.9) |
| Valerate | — | 0.2 (0.1) | 4.5 (0.5) | 3.6 (0.8) | 4.2 (0.2) | 2.8 (0.1) | 4.3 (0.9) |
| Iso-caproate | — | 0.1 (0.1) | 0.6 (0.3) | 0.2 (0.2) | — | — | 0.9 (0.3) |
| Caproate | — | 0.3 (0.2) | 1.4 (0.5) | 1.7 (0.8) | 1.7 (0.6) | 0.3 (0.2) | 1.5 (0.3) |
| Lactate | 2.0 (1.2) | 13.5 (5.5) | 4.5 (1.4) | 3.1 (1.7) | 3.5 (2.2) | 3.1 (2.1) | 1.5 (1.5) |
| Succinate | 3.7 (1.3) | 8.3 (3.2) | 0.9 (0.2) | 3.1 (0.9) | 1.7 (0.4) | 1.9 (0.6) | 2.1 (1.0) |
| N† | 6 | 6 | 6 | 6 | 5 | 4 | 5 |

*mmol/kg contents (± 1 SEM); †number of samples. In some cases areas of the large intestine did not contain enough material for analysis; ‡none detected (less than 0.1 mmol/kg).

$r = -0.60$, $p < 0.01$, $n = 24$), low pH and high SCFA going together.

Detailed composition of the organic anion in human intestinal contents is given in Table 2. Acetate was the principal anion followed by propionate and butyrate in approximately equal amounts. Small quantities of the branched chain fatty acids iso-butyrate and iso-valerate were present, as were small amounts of lactate and succinate. Little or no oxaloacetate, oxalate, malate or fumarate were detected in most samples, however (less than 1.0 mmol/kg).

Short chain fatty acid concentrations in portal, hepatic, and peripheral venous blood are given in Table 3. All three anions were detected in portal blood although concentrations were about 1/1000th less than in colonic contents. Total concentrations were 375 $\mu\text{mol/l}$ in portal blood falling by 60% in hepatic blood to 140 $\mu\text{mol/l}$, and then a further fall in peripheral blood to 79 $\mu\text{mol/l}$. Marked differences were seen in molar ratios (Table 4) of the three major short chain fatty acids amongst the various regions where they were found. In colonic contents the molar ratios of acetate:propionate:butyrate are 57:22:21 for all regions, despite a significant fall in individual anions between right and left colon. In portal blood, however, the relative amount of butyrate falls to only 8% whilst transport through the liver reduces propionate from 21 to 12% of the total. In peripheral blood only acetate is found in measurable quantities.

Discussion

There are major difficulties in establishing the digestive function of the large intestine, especially the caecal area, because of its inaccessibility. In order to gain access to the colon and the blood draining the gut in people who were eating normally, we decided

to study sudden death victims – that is, people who without any antecedent symptoms of ill health requiring medical attention died suddenly. In the UK such deaths are principally from road traffic accidents or

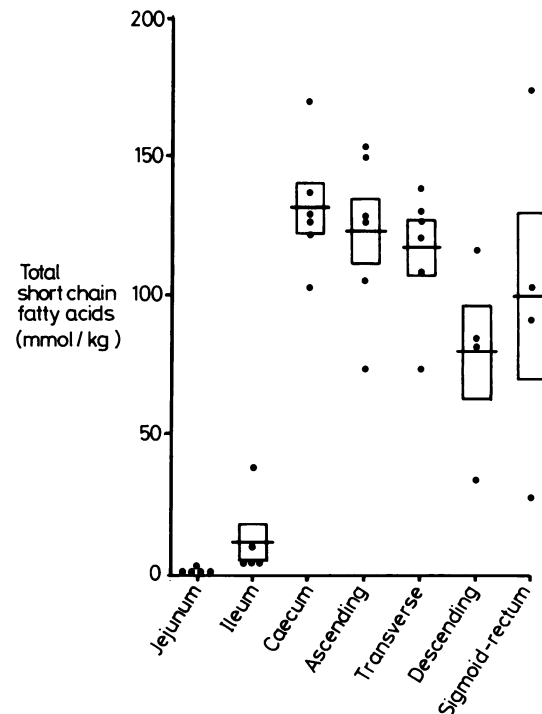


Fig. 4 Total short chain fatty acid concentrations (mmol/kg) in intestinal contents of six subjects as described in Fig. 3. J=jejunum; I=ileum; C=caecum; A=ascending; Tr=transverse; D=descending; S/R=sigmoid/rectum. Box=Mean+1 SEM.

Table 3 Short chain fatty acid anions in human blood ($\mu\text{mol/l}$)

| Case no | Portal | | | Hepatic | | | Peripheral | | |
|-----------|--------|-----|----|---------|----|----|------------|----|----|
| | A | P | B | A | P | B | A | P | B |
| 1 | 108 | 17 | 14 | 32 | 2 | 2 | 28 | 1 | 1 |
| 2 | 295 | 142 | 64 | 78 | 10 | 4 | 19 | 1 | 1 |
| 3 | 289 | 38 | 28 | 116 | 14 | 9 | 80 | 4 | 2 |
| 4 | 231 | 49 | 15 | 103 | 14 | 12 | 79 | 5 | 4 |
| 5 | 404 | 194 | 35 | 239 | 69 | 32 | 146 | 13 | 12 |
| 6 | 220 | 90 | 16 | 124 | 18 | 11 | 68 | 6 | 3 |
| \bar{x} | 258 | 88 | 29 | 115 | 21 | 12 | 70 | 5 | 4 |

A = Acetate; P = Propionate; B = Butyrate.

coronary heart disease, and an autopsy is usually ordered by the coroner. A potential disadvantage of the material obtained from such cases is that post mortem changes may make interpretation of the results difficult. To gauge the extent of this change we tried to reproduce the conditions of sampling in mature pigs who were allowed free access to their usual diet until the time of slaughter. The principal change we expected was a continuation of fermentation with resultant increasing concentrations of SCFA and associated fall in pH. No change in pH was seen with time nor in SCFA concentrations in the ileum, mid colon or rectum. There was, however, a rise in SCFA concentration in the caecum over the first three hours after death which was significant statistically, although the trend over four hours was not. No associated change in pH was seen, however, and, as Figure 2 shows, the differences between regions in SCFA levels were much greater than any changes seen with time. The absolute values for both pH and SCFA concentrations observed in the pigs were within the wide range reported from other studies of pigs.¹⁷⁻¹⁹

A time limit of four hours was chosen for these studies as the minimum realistic interval between death and the completion of the necessary authorisations to proceed with and complete an autopsy. *In vitro* studies of microbial fermentation indicate that significant SCFA production may occur over this time at 37°C²⁰ but the rapid cooling of bodies after death, the complete absence of colonic motility and the poor availability of carbohydrate substrates may explain the relative slowness of these changes seen in the pig, and probably therefore in man. In studies of pigs Moore *et al.*²¹ found that samples taken from the intestine at four hours after death were bacteriologically indistinguishable from specimens taken from the same animal at the time of death. Nevertheless post mortem fermentation probably does occur and the present values must be viewed in this light. The pig studies, and those comparing human faecal SCFA and values from the sigmoid/rectum in this study,

Table 4 Molar ratios* of acetate, propionate and butyrate in human intestinal contents and blood (n=6)

| | Acetate | Propionate | Butyrate |
|-----------------|---------|------------|----------|
| Right colon | 57 (2) | 22 (2) | 21 (2) |
| Left colon | 57 (1) | 21 (1) | 22 (1) |
| Portal vein | 71 (4) | 21 (4) | 8 (1) |
| Hepatic vein | 81 (2) | 12 (2) | 7 (1) |
| Peripheral vein | 91 (1) | 5 (2) | 4 (1) |

*% of total acetate + propionate + butyrate (± 1 SEM).

however, suggest that changes with time are unlikely to be great and to obscure the larger differences between regions in the colon, nor to alter the magnitude of overall levels of these fatty acids unacceptably.

In man these studies provide clear evidence of fermentation occurring in the large intestine, especially the right side. The progressive decline in total SCFA concentrations coupled with the rise in pH as contents proceed distally suggests that the amount of substrate available for fermentation is limiting these processes to the caecum and ascending colon predominantly. Total concentrations of acetate + propionate + butyrate in the sigmoid/rectal area (87 mmol/kg) are similar to those found in faeces by others which range from 72–83 mmol/kg^{12,22-24} and 66 mmol/kg in previous studies of our own.²⁵ The lower concentration of SCFA found in faeces when compared with that of the right colon limits the extent to which faecal data can be used to interpret changes in fermentation occurring in the colon. Moreover, SCFA are rapidly absorbed from the human colon^{26,27} so it is hardly surprising that most studies of the effect of diet on faecal SCFA levels in man have shown little if any change.^{12,22,25,28}

The pH of colonic contents *in vivo* has been measured using a pH sensitive radiotelemetry device by Bown *et al.*²⁹ and Patil *et al.*³⁰ in healthy subjects. Right colonic pH in these studies were 6.0 and 6.36 respectively compared with a mean (arithmetic) of 5.9 in the present study. Both the present study and those using the radiopill show a significant change in pH from right to left colon of about 1 unit. We have shown that this change in pH is associated with a change in SCFA concentrations, these being higher in the caecum where pH is low, and presumably fermentation is most active, and lower in the sigmoid/rectum where pH is more alkaline. The relative acidity of the caecal area, and its relation to carbohydrate fermentation,^{29,30} may be important in the control of microbial enzyme activity such as bile acid 7- α -dehydroxylase.³¹ The sharp fall in pH from terminal ileum (6.3) to caecum (5.6) in the present studies confirms what was suspected from radiotelemetry studies but could not be conclusively

established because of doubts about the position of the radiopill.

Small amounts of the branched chain fatty acids isobutyrate and isovalerate/2-methyl-butyrate were present in most samples. These fatty acids originate from the catabolism of the amino acids valine and leucine/isoleucine respectively. In separate studies we have shown that human colonic bacteria are capable of protein breakdown and amino acid fermentation.^{32,33} The presence of these fatty acids confirms the occurrence of proteolysis in man, the extent of which may be critically important in determining large bowel function particularly in those people living on diets low in fermentable carbohydrate.

All three major SCFA are present in portal blood at concentrations several times greater than peripheral venous blood indicating the gut as a major source of these fatty acids. Only one previous attempt to measure short chain fatty acids in human portal blood has been published, that of Dankert and colleagues,³⁴ who found total (acetate+propionate+butyrate) SCFA concentrations of 155 $\mu\text{mol/l}$ in five subjects undergoing surgery for gall bladder disease. This is lower than the present values ($378 \pm 70 \mu\text{mol/l}$) but gall bladder surgery patients would have been starving for at least 12 hours before surgery whereas in the sudden death victims there was evidence of food in the small intestine in all cases (average contents 291 g). The SCFA values shown in Table 2 are much lower than have been reported for other species such as the rabbit, which is a hindgut fermenter ($5\text{--}6 \text{ mmol/l}$)³⁵ and the rat ($1\text{--}2 \text{ mmol/l}$)^{36,37} and somewhat lower than the pig ($0.4\text{--}1.0 \text{ mmol/l}$).^{18,19,38} The average British diet, however, contains much less carbohydrate which reaches the large intestine than that present in the diet of these other species. Moreover the considerable difficulties in measuring SCFA in blood¹⁶ make comparisons less easy to interpret. Our method is virtually identical to that used by Topping *et al*²⁰ in the pig, however, and shows clearly lower levels in the human portal vein, in fact lower levels than for any other species for which there are data at present.

Hepatic vein SCFA concentrations are only 39% of those in portal blood. As portal blood provides two thirds to three quarters of total hepatic blood flow in man^{39,40} there is clearly significant uptake of these anions by the liver. Further uptake occurs in peripheral tissues because peripheral venous blood levels are again lower at 53% of hepatic venous blood. In previous studies¹⁶ of healthy individuals we have shown forearm arteriovenous differences in blood acetate of $64 \mu\text{mol/l}$ (arterial blood 126 ± 13 , venous blood 61 ± 7 , $n=7$).

A pronounced change in molar ratios of the SCFA

occurs between the gut lumen and portal blood with the proportion of butyrate falling from 21 to 8%, whilst acetate rises from 57 to 71% of the total. One explanation for this fall in the relative amount of butyrate could be greater uptake by the colonic mucosa of this anion than of acetate and propionate. It has been shown in man that the colonic epithelium actively metabolises butyrate⁴¹ whilst in the guinea pig colonocyte⁴² butyrate is metabolised in preference to acetate and propionate. Arterial blood supplying the gut contains predominantly acetate,¹⁶ however, so when short chain fatty acids are absorbed from the gut into this blood a fall in the proportion of butyrate would occur, without any epithelial metabolism. Nevertheless, the data suggest some selective uptake by the mucosa.

From these data, particularly the amounts of SCFA in portal blood, it must be inferred that substantial carbohydrate breakdown is occurring in the large intestine. Other studies of the microflora of the human large gut indicate that more fermentation is occurring than can be accounted for simply by the breakdown of dietary fibre alone.⁴³ These differences are probably caused by starch. Furthermore the presence of branched chain fatty acids in colonic contents indicates that protein breakdown is also occurring. Together the evidence points to the colon having a much larger role in digestive physiology than has previously been attributed to it and one that might be substantial in people living on diets much higher in starch and fibre than those which are average in Britain.

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