Microbial digestion of complex carbohydrates in man

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There is now substantial evidence that a fermentative process similar to that occurring in non-ruminant herbivorous and in omnivorous species takes place also in the human large intestine. Its metabolic significance, however, is by no means certain. The principal fermentable substrate available in the human colon is probably the polysaccharide fraction of plant cell walls, currently referred to as dietary fibre. In the UK this amounts to only 15–20 g/d (Bingham *et al.* 1979) and in many Western countries rarely exceeds 30 g on average (Bingham & Cummings, 1980). This represents only 3–4% of dry matter intake which, when contrasted with the 10–20% of fermentable carbohydrate in the diet of species such as the pig or horse, makes it easy to appreciate why this property of the gut has been neglected in man.

A fairly limited view of colonic function has traditionally been taken by medical science. The large intestine is usually thought of as an organ for the conservation of salt and water and for the orderly disposal of waste products from the body. The notion that it might be an important metabolic structure with influence outside its walls has not been seriously considered in recent years, with occasional notable exceptions (Wrong, 1971). Furthermore, the ability of the human species to survive in reasonable health after removal of the colon has confirmed in many their belief of its rudimentary function and importance (Ritchie, 1971; McNeil *et al.* 1982). Of the occurrence of colonic fermentation in man, however, there can be little doubt.

Occurrence of cell-wall polysaccharide breakdown in man

For more than 100 years it has been known that 'fibre' was digested in man. Around the turn of the century considerable interest was being taken in cellulose breakdown by Rubner and others (see review, Mangold, 1934). Of more recent studies that of Williams & Olmsted (1936) is one of the most notable. They reported dietary experiments with three medical students to whom they gave cell-wall preparations from carrot, cabbage, peas, bran, lucerne (*Medicago sativa*) and also cellulose and agar. With most of the preparations only 20% of the hemicellulose (non-cellulosic polysaccharide; NCP) fraction fed could be recovered in the stools although more than 60% from bran survived breakdown. Cellulose appeared to be less digestible but even so about 50% disappeared during passage through the gut.

Since the 1930s, methods for the measurement of plant cell-wall polysaccharides have improved considerably and this has led to a greater understanding of their metabolism, but the over-all conclusion, that substantial breakdown occurs,
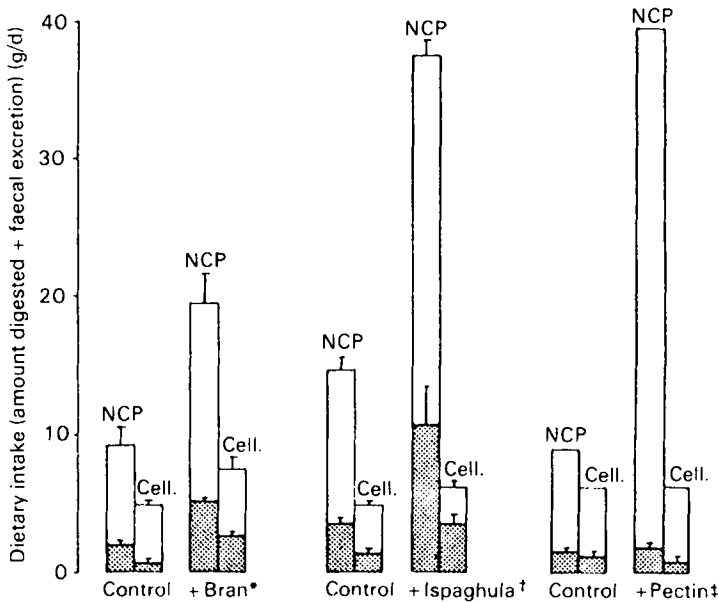
Fig. 1. Dietary intake (amount digested (□) plus faecal excretion (▨)) of the principal cell-wall polysaccharides, cellulose (Cell.) and non-cellulosic polysaccharides (NCP), in three groups of healthy human volunteers given typical UK diets under controlled conditions (Control) and then the same diet with the addition of either bran, ispaghula or pectin. From *Southgate *et al.* (1976), †Prynne & Southgate (1979), ‡Cummings *et al.* (1979).

remains. Fig. 1 shows the results of three recent studies of groups of healthy adults in which dietary cellulose and NCP intakes and faecal excretion of these polymers have been measured using the techniques of Southgate (1976). Both the cellulose and NCP from the control diets (which were typical mixed UK diets) are extensively degraded. The cell-wall polysaccharides in added bran are partly degraded, in ispaghula substantially and the pectin completely so.

The relative resistance of bran to digestion has been noted by others (Heller *et al.* 1980; Stephen & Cummings, 1980a). Its poor digestibility in human dietary studies is one example of the wide variability that occurs in fermentable polysaccharide breakdown. In ruminant nutrition this variability has important physiological, and economic, implications but in man it has received little attention. A number of chemical and physical characteristics of these polysaccharides may influence their breakdown including, for example, the extent of lignification of the cell-wall polymers (bran cell-walls being one of the most lignified of human foods), their water solubility (generally water-soluble polysaccharides such as pectin are highly digestible), associated silica and cuticular substances (which are thought to inhibit breakdown in the rumen), particle size (the finer the particle the more readily digestible), molecular structure, the level of intake, food processing (for example, heating and drying) and possibly previous diet. Host factors affecting breakdown will include the type of gut microflora and transit time (see review, Cummings 1982).

Site of breakdown

The evidence, therefore, that the complex carbohydrates of the plant cell-wall are broken down in the human gut is quite clear. The principal site of this breakdown is widely assumed, probably correctly, to be the large intestine, although in fact little experimental work has been done to document this. One method of obtaining evidence for the site of breakdown of these polysaccharides is to feed them to people who have an ileostomy. These are subjects whose large intestine has been removed, usually on account of inflammatory bowel disease, and an exit to the terminal ileum made on the anterior abdominal wall. Sandberg *et al.* (1981) gave 16 g bran daily to each of nine subjects with an ileostomy. They were able to recover about 88% of the hemicelluloses in the ileostomy effluent. Although bran is relatively poorly digested during passage through the entire gut the almost complete recovery of the more digestible of its cell-wall polysaccharides from the terminal ileum indicates that any breakdown which does occur is probably in the colon. Holloway *et al.* (1980), however, were able to recover only 35% of the hemicelluloses from mixed diets given to female ileostomists and 17% from males, suggesting substantial small bowel degradation. Similarly 15% of pectin in women and 47% in men could not be recovered in a further series of studies (Holloway *et al.* 1983). In contrast, Werch & Ivy (1941) had found little evidence for pectin breakdown in the small bowel of man. Further studies by Holloway *et al.* (1978), using the same ileostomy model as for their pectin and hemicellulose experiments, had shown no significant degradation of the cellulose and hemicellulose of bran. Work by McLean-Ross *et al.* (1981) in which gum arabic was incubated with gastric juices in vitro has not shown evidence of significant breakdown of this polysaccharide, whilst in our own studies (H. N. Englyst and J. H. Cummings, unpublished results) with cereals, complete recovery from ileostomy effluent of cell-wall polysaccharides has been achieved. The contrasting results in these studies are probably a reflection of the methodological difficulties both in the use of the ileostomy model and in accurate measurement of dietary polysaccharides especially in the presence of gut contents. That the colon is the main site of breakdown of these polysaccharides can be deduced from the absence of digestive enzymes in the human small bowel known to hydrolyse plant cell-wall polysaccharides, of a significant microflora and of signs of fermentation such as appreciable concentrations of short-chain fatty acids in this part of the gut.

The human colon contains a substantial mixed culture of bacteria estimated at 10^{10}–10^{11}/g colonic and faecal material, 99% of which are anaerobes (Moore *et al.* 1978). Most of these organisms are saccharolytic, that is they derive their energy primarily from carbohydrate and its derivatives (Miller & Wolin, 1979). Many species isolated from the human colon have been shown to ferment hemicellulose, cellulose and pectins. The considerable work done by Salyers and her colleagues in this field has been recently reviewed (Salyers, 1983).

The presence of acetic, propionic and butyric acids in the colon is clear evidence of fermentation. These acids are the principle anions in human colonic contents and faeces at concentrations of 60–100 mmol/kg and molar ratios for acetate:

propionate:butyrate of about 60:25:15 (Cummings, 1981). These are similar ratios to those found in species where fermentation is known to be an important part of normal digestive physiology. Other end-products of fermentation, the gases carbon dioxide, hydrogen and methane, are also produced in man. Their excretion per rectum may on occasion be a source of social discomfort but since hydrogen and methane are not produced by mammalian cells they provide further evidence for the fermentative process.

The balance of evidence therefore is in favour of fermentation, the anaerobic breakdown of carbohydrate, occurring in the human colon. In fact this is probably the principal metabolic event in this organ and as such is unique to the human body. What is its significance? In terms of the metabolism of the microflora it is considerable.

Fermentation and the microflora

Bacteria in the colon metabolize carbohydrate, and possibly other substrates, in order to obtain energy for growth and for the maintenance of cellular activity. There are, however, considerable difficulties in documenting microbial metabolism in man. Most studies of human colonic microflora have relied on examination of faeces using the classical techniques of taxonomic bacteriology. The use of faeces for this purpose is understandable in view of the problems in obtaining reliable samples from the colonic lumen but raises the question of how representative are faecal bacteria of the microflora in other regions of the colon?

In separate studies Moore et al. (1978) and Croucher et al. (1983) have examined the flora in different areas of the colons of the victims of sudden death. Both groups conclude that there are no detectable regional differences using classical methods, within the colon of man. Moreover the flora associated with the mucosal surface do not seem to be significantly different from those in the lumen of the bowel. Accepting, therefore, the possible limitations in the use of faecal flora as a guide to colonic flora, a number of studies of the effect of diet may be noted. No change in total bacterial counts nor in the numbers or distribution of species have been found in healthy subjects given bran (Drasar et al. 1976; Fuchs et al. 1976), bagasse (Walters et al. 1975), guar gum (Drasar & Jenkins, 1976) and pectin (Doyle et al. 1981) when compared with a control low-fibre diet. This apparent stability of the colonic microflora to dietary change, not only in fibre content but also to changes in fat and protein, has led to the suggestion that the methods of classical bacteriology are not sensitive enough to detect differences and that alternative methods are required.

Studies of the 'metabolic' activity of micro-organisms in faeces by measurement of faecal microbial enzymes have, to date, been inconclusive (Cummings, 1983) but changes have been observed in the microflora using a gravimetric method derived from animal nutrition for determining microbial mass in gut contents (Stephen & Cummings, 1980b). Other chemical markers of microbial activity such as DNA, RNA, ATP and diaminopimelic acid have not been used in man to any significant extent as yet.

Using the gravimetric method it has been observed in man that feeding fermentable carbohydrate stimulates microbial growth and increases microbial cell excretion in faeces. Fig. 2 shows the effect on daily faecal microbial cell mass excretion of three different plant cell-wall preparations given to groups of healthy subjects. These studies were carried out with volunteers living in a metabolic unit and eating controlled diets containing about 20 g plant cell-wall polysaccharides from mixed sources daily for 3 weeks and then for a further 3-week period the same diet with the addition of between 14 and 19 g polysaccharide from cabbage, carrot or bran. It is evident from Fig. 2 that each type of fibre leads to a significant increase in the daily excretion of microbial material, the increase being 4·8 g (33%) for cabbage, 3·8 g (25%) for carrot and 2·3 g (15%) for bran. The increase with bran is less than that for the other two, probably because the cell-wall polysaccharides in bran resist breakdown by the microflora to a much greater extent than those in cabbage or carrot. Of the 18·3 g cabbage fibre fed daily only 1·6 g/d could be recovered in faeces, i.e. 16·3 g/d was fermented (89%); for carrot 17·4 g/d (90%) was fermented but for bran only 6·4 g/d could not be recovered (45% fermented). Providing fermentable substrate to colonic bacteria therefore leads to a stimulation in their growth and metabolic activity.

Control

The control of fermentation is of considerable significance in the over-all function of the human large intestine. The amount of substrate available is one important factor determining microbial cell growth but because of the difficulties in studying bacterial metabolism in vivo only approximate values are available for the flow of substrate through the complex and varied routes of fermentation in man (Cummings, 1981). One control over fermentation which has been well

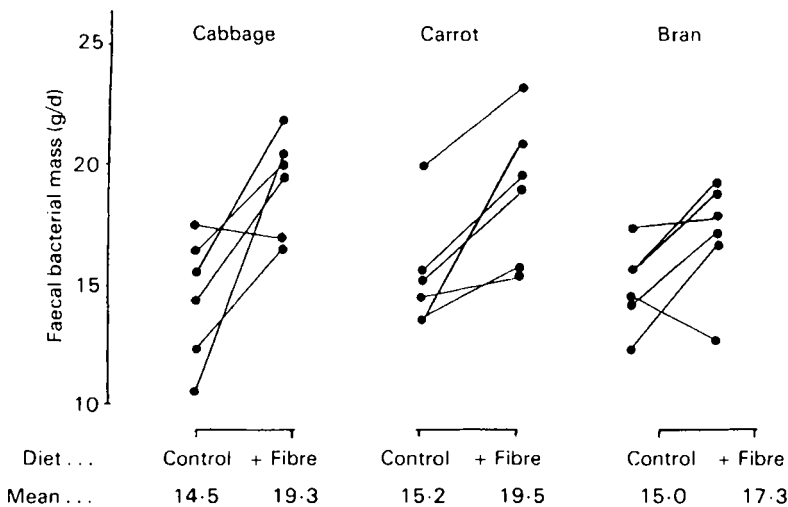| Diet ...	Control	+ Fibre	Control	+ Fibre	Control	+ Fibre
Mean ...	14·5	19·3	15·2	19·5	15·0	17·3

Fig. 2. Mean daily faecal excretion of microbial solids in three groups of healthy volunteers given a typical UK diet containing about 20 g cell-wall polysaccharides for a 3-week period and then the same diet with the addition of 18·3 g cell-wall polysaccharides from cabbage, or 19·3 g from carrot or 14·2 g from bran. From Stephen & Cummings (1980a) and J. Banwell (unpublished results).

documented in vitro is the dilution rate or turnover time (Hespel, 1979; Hespel & Bryant, 1979). In the studies of Isaacson *et al.* (1975), mixed populations of anaerobic rumen bacteria were grown in vitro in continuous culture with glucose as the only energy source. The yield of microbial cells per mol glucose fermented (efficiency of conversion) increased from 42 g cells/mol at a dilution rate of 0·02/h (turnover time of 50 h) to 84 g cells/mol glucose at 0·12/h (turnover time 8·3 h). On a simple weight basis the yield of microbial matter ranged from 230 to 460 g/kg carbohydrate fermented. Salyers and colleagues (Salyers *et al.* 1981; Kotarski & Salyers, 1981) have shown, using very similar techniques but with single strains of human colonic bacteroides, that cell yields are of the same order, 30–40%, when either glucose or an arabinogalactan is the primary energy source. At a turnover time of 32 h, cell yields were 60 g/mol glucose rising to 75 g/mol glucose at 8 h. In these studies the more rapid the turnover the greater the efficiency of fermentation due to diminished requirements by the bacteria for maintenance energy (Owens & Isaacson, 1977).

In man turnover of gut contents, or transit time as it is more usually called, has been measured by both continuous- and intermittent-marker methods and averages 48–72 h in normal subjects living in the UK (Cummings, 1978; Wyman *et al.* 1978; Eastwood *et al.* 1982). Of this about 40–60 h will be spent in the large intestine. Stephen & Cummings (1981) have taken a group of eight volunteers living on a controlled diet and measured daily faecal microbial cell output during a basal period and then after speeding up transit pharmacologically with the drug Senokot or slowing it down with codeine phosphate. When transit was speeded up from 64 to 35 h, microbial solids excretion rose from 16·5 to 20·3 g/d and when it was slowed from 47 to 88 h, microbial solids fell from 18·9 to 16·1 g/d, results which suggest that transit may affect the efficiency of microbial metabolism in man also. Using the values shown in Fig. 2 it is possible to derive microbial cell yields for man in vivo since the daily output is known as is the loss of fermentable carbohydrate in the gut, assuming that the cell-wall polysaccharides from the three plant sources are the only additional substrate available to the bacteria. Microbial cell yields (g cells/kg carbohydrate fermented) are for cabbage 290, carrot 220 and bran 360. These values, despite the assumptions that have to be made in doing these calculations, are surprisingly similar to those derived during in vitro studies, and confirm the close qualitative parallels there are between the fermentative patterns of the human hind-gut and those of other animals including the ruminant.

Importance of fermentation

Given that the breakdown of complex carbohydrates in the human large intestine by the microflora is a normal part of colonic function, what is its significance? The quantitative importance of hind-gut fermentation is the subject of some debate. The major end-products are short-chain fatty acids which are rapidly absorbed from the human colon (McNeil *et al.* 1978; Ruppin *et al.* 1980) and therefore contribute to the host's energy metabolism. In the pig Partridge (1982) argues that the contribution to metabolizable energy of absorbed short-

chain fatty acids is insignificant. In man if the only substrate for fermentation is dietary fibre then only 20 g/d on average are broken down in subjects living on UK-type diets. Assuming that short-chain fatty acid absorption yields about 70% of the energy available had the carbohydrate been digested in the small intestine (Smith & Bryant, 1979) the net energy yield is of the order of only 225 kJ or 2·5% of daily energy intake, an insignificant amount. If, however, there is more carbohydrate breakdown in the colon than just fibre from sources such as starch or mucus, and values from studies of microbial cell output in faeces and of breath hydrogen excretion after starch ingestion would suggest there is, then the contribution to energy metabolism will be proportionately greater. In countries where fermentable carbohydrate intakes are of the order of 100 g/d or more (Bingham & Cummings, 1980) short-chain fatty acids could be of considerable importance energetically.

Fermentation, by stimulating microbial growth, increases the requirements of micro-organisms for nitrogen. When dietary fibre intakes are increased in man faecal N excretion also increases (Calloway & Kretsch, 1978; Kelsay *et al.* 1978). Much of the extra faecal N in both animals and man is due to increased microbial N excretion (Mason, 1969; Mason & Palmer, 1973; Stephen & Cummings, 1979). The main source of N in the colon is ammonia derived either from urea or protein. In the absence of active fermentation much of this ammonia is absorbed and passes to the liver where it is converted to urea. Vince *et al.* (1977) have shown, using human colonic microflora incubated anaerobically in vitro, that ammonia concentration falls when lactulose, a disaccharide, is added to the medium. In patients with cirrhosis of the liver, lactulose (which escapes digestion in the human small bowel) brings about a two to threefold increase in faecal N excretion and a decrease in urea production and urea pool size (Weber, 1979). Faecal ammonia levels fall in healthy human volunteers given fermentable carbohydrate (Cummings *et al.* 1981). Microbial breakdown of complex carbohydrates therefore leads to measurable changes in colonic N metabolism. The lowering of colonic ammonia concentration and absorption may be important in protection against carcinogenesis (Visek, 1972).

Short-chain fatty acids may have a significance other than simply that of their potential energy. Both the rumen and colonic epithelium metabolize short-chain fatty acids, especially butyrate and propionate. In the rumen these acids stimulate normal cell growth (Sander *et al.* 1959) and in man Roediger (1980*a*), using isolated colonic epithelial cells, has shown that n-butyrate is used for aerobic metabolism and suppresses glucose oxidation by 50%. In further studies using the epithelial cells from patients with ulcerative colitis in remission, Roediger (1980*b*) has gone on to show that butyrate oxidation is reduced and suggests that this is a specific defect in metabolism which may be important in the aetiology of this common condition.

Butyrate also has antineoplastic properties. In cancer-cell studies it prolongs doubling time and reduces the rate of growth of human large bowel cancer cells (Kim *et al.* 1982; Kruh, 1982). It affects a wide range of cellular enzymes, may

stabilize chromatin structure during cell division and suppress cell proliferation. Propionate, however, seems to be less important in maintaining the integrity of the colonic epithelium but is rapidly cleared by the liver where it may affect gluconeogenesis (Anderson & Bridges, 1982).

Potentially there are many other aspects of metabolism which may be affected by the breakdown of polysaccharides by the microflora in the colon. Complex carbohydrates are probably the main energy source for the fermentative bacteria so indirectly dietary intake of these polymers could affect the immune system, resistance to gut infection, steroid, mucus and enzyme metabolism (Gustafsson & Eriksson, 1970; Gustafsson, 1982). What is not yet certain is the extent to which these aspects of metabolism are related to diet through fermentation and could be modified by changing food intakes.

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