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Definitions and history

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Definitions

This book is primarily concerned with the acetic, propionic and *n*-butyric acids that are generated by microbial fermentation within the digestive tract. They are substances which present a slight problem in terminology. Originally they were described as 'volatile fatty acids' (VFA), the word 'volatile' being used because they were measured by steam-distillation, after acidification, of intestinal contents. Now that steam-distillation has been superseded by gas–liquid chromatography, this terminology has been largely abandoned, and they are usually called 'short-chain fatty acids' (SCFA). The term 'fatty acid' is misleading in several ways. It is in general use by organic chemists, because the longer members of the series, particularly stearic and palmitic acids, are present in the triglycerides of natural fats. But SCFA are biochemically more closely related to carbohydrates than to fats, some of them are not constituents of natural fats, and they are not 'fatty', as the layman envisages the term, as they are completely miscible with water. If we wished to be absolutely precise, we would describe the SCFA that interest us as 'saturated unbranched alkyl monocarboxylic acids of 2–4 carbon atoms'. Certainly 'SCFA' is a more convenient term, despite its faults.

The digestive tract contains other carboxylic acids of small molecular size, usually in much smaller amounts. By general agreement, those with further substitutions – dicarboxylic acids (e.g. succinic), carbonyl acids (e.g. pyruvic) or hydroxy-acids (e.g. lactic) – are not described as 'SCFA', nor are they sufficiently volatile to be measured in the same gas–liquid chromatographic systems as the cardinal three SCFA. There is less agreement on what length the molecule of an unsubstituted acid should be to qualify as 'SCFA' – are acids with five, six or seven carbon atoms too long, and is formic acid with its single carbon atom too short to be included? These questions tend to disappear in practice, for these unsubstituted acids (formic, valeric, hexanoic,

heptanoic and the branched-chain isomers of butyric and the last two acids), although easily measured in the chromatographic systems that record the main three SCFA, are present in digesta in such small amounts that most workers either disregard them as 'SCFA', or specify them separately. In practice, therefore, the term 'SCFA' is usually applied in a restricted sense to acetic, propionic and *n*-butyric acids, the three organic acids that are most abundant in the digestive tract.

In physiological terms these three SCFA are moderately strong acids, with p*K* values of about 4.8. Intestinal contents are more alkaline than this, so within the intestine SCFA are predominantly present as negatively charged *anions*, and not as free *acids*. But by convention many workers in the field refer to SCFA as *acids* even when describing their physiological properties as *anions*. SCFA are not volatile when ionized (hence the need to acidify samples to liberate free acids when they are measured by steam-distillation or gas–liquid chromatography), as can be realized by comparing the smell of a solution of sodium acetate with one of the acetic acid.

Ruminant studies

Most of the work on SCFA is performed by comparative zoologists and veterinary physiologists, and not (with a few notable exceptions) by human physiologists or clinical gastroenterologists. Emphasis on animals rather than humans, and particularly on herbivorous animals, has been a feature of work on the physiology of SCFA since it began in the nineteenth century, when the importance of microbial fermentation within the rumen to the nutrition of cattle and sheep was first realized and provided a strong commercial inducement to veterinarians to work in this field. In contrast, clinicians and alimentary physiologists working mainly with humans have tended to regard intestinal bacteria as unfortunate contaminants, of little metabolic significance for their hosts. Despite a recent awakening of interest among physicians, the subject is relatively neglected even today, as shown by meetings of the American Gastroenterological Association and the British Society of Gastroenterology: out of a grand total of 3738 research abstracts submitted to the two societies at their 1992 annual meetings, only 40 were in any way concerned with SCFA, an unimpressive 1.1%. Ignorance of veterinary (particularly ruminal) discoveries is still widespread among research gastroenterologists, and has led to some wasteful reduplication of effort, by myself among others.

The 1962 and 1966 monographs by Blaxter and Hungate, respectively, are useful starting points for those interested in the function of the rumen. In his introduction on the history of his subject, Hungate pointed out that some

basic facts were known as long as 160 years ago; in 1831 Tiedemann & Gmelin found evidence of acetic and butyric acids in rumen contents, and a year later Sprengel, in his two-volume textbook, *Chemistry for Landowners, Foresters and Accountants* (my translation), pointed out that plant materials decomposed in the rumen to yield volatile breakdown products (Sprengel, 1832). In 1888 Tappeiner found that cellulose incubated with rumen contents was fermented to acetic and butyric acids. Protozoa, chiefly highly motile ciliates, were found to be abundant in the rumen as early as 1843 (Gruby & Delafond), and were subsequently shown to be involved in the fermentation of several forms of fibre, including cellulose, though they also feed by ingesting ruminal bacteria. These ciliates are of particular interest to zoologists, as most varieties have no close similarity to existing free-living protozoa, which suggests that they have evolved over millions of years in close association with the evolution of their hosts. However, the main agents of fibre fermentation are the abundant anaerobic bacteria in the rumen. Hungate explained the energy yield of microbial fibre digestion, in a form comprehensible to non-biochemists, by pointing out that the carbon in carbohydrates (—CHOH— groups), is at an intermediate stage of oxidation, and the anaerobic rearrangement, by which some carbon atoms become more oxidized (CO_2 and —COOH) and others more reduced (CH_4 and —CH_2—), produces energy that is available to micro-organisms for the synthesis of high-energy molecules such as ATP. The main end-products of these reactions are methane, carbon dioxide, and the three cardinal SCFA already defined, the actual proportions of these metabolites depending on the type of fibre digested and the organisms responsible. The three SCFA derived from plant fibre are all metabolized by the ruminant host, and calculations of the proportion of the original polysaccharide energy available to the host SCFA have produced estimates of 65–75% (Marston, 1948; Blaxter, 1962; Hungate, 1966). In addition to SCFA, some other organic acids, including lactic, succinic and formic acids, are produced in much smaller amounts, along with ethyl alcohol and hydrogen. Plant fibre is the predominant but not the only ruminal source of SCFA, which are also derived from microbial metabolism of proteins. The branched-chain amino acids from this metabolism give rise to the isobutyrate and isovalerate found in the rumen. Fats do not figure greatly in ruminal fermentation, but glycerol is partially converted to propionic acid, and unsaturated fatty acids are largely hydrogenated to their saturated analogues.

Non-ruminant studies

The importance of microbial fermentation of plant fibre to non-ruminant mammals has only slowly been realized. In 1885 Louis Pasteur suggested that

the presence of bacteria in the alimentary tract was essential for the life of rabbits and guinea-pigs, and although this suggestion was subsequently disproved by the successful rearing of germ-free specimens of these animals, their shortened survival supported the principle that the intestinal flora was important for normal nutrition. The anatomical studies of Flower (1872) and Mitchell (1905) led to the appreciation that all predominantly herbivorous animals have, at some site in their digestive tract, an expanded fermention chamber that is often sacculated or subdivided by septa (McBee, 1977; Wrong, Edmonds & Chadwick, 1981; Clemens & Maloiy, 1982; Argenzio & Stevens, 1984). A particularly useful source is the 1988 monograph by Stevens on comparative aspects of the vertebrate digestive tract, which points out that of the 20 mammalian natural orders that exist at the present day there are 11 containing species that subsist largely on the fibrous parts of plants. Similar adaptations to such a dietary regime have sometimes developed in species that are only distantly related genetically; conversely, animals that are closely related may have very different alimentary adaptations for fibre fermentations. The most fundamental distinction is between herbivores with a fermentation chamber in the foregut, and those in which it is in the hindgut. Ruminants are the most obvious examples of the former, but the stomach is modified into the form of a fermentation chamber in many herbivores that do not ruminate, such as the camel, llama, hippopotamus, many marsupials and some foliage-eating monkeys and sloths. The herbivores with a hindgut fermentation chamber, in the form of an enormously capacious caecum or colon, include the perissodactyla (the horse, tapir and rhinoceros), lagomorphs (rabbits and hares), elephants, most rodents, and many primates. Some of these hindgut fermenters practise coprophagy, which ensures that bacterial metabolites arising in the large intestine have a further opportunity to be absorbed by the host. Many herbivores, particularly some species of pig, have well-developed fermentation chambers in both foregut and hindgut. The hyrax (probably, in Chapter 11 of the book of Leviticus, the coney that Jehovah forbade the children of Israel to eat) is unique in possessing fermentation chambers at both sites and a further chamber in the midgut. Omnivorous animals such as humans and the rat have a moderately well-developed fermentation chamber in the large intestine, without a fermentation chamber in the foregut, whereas purely carnivous animals such as the dog and ferret have no obvious fermentation chamber at any level, though their large intestine contains bacteria that have the ability to ferment plant fibre. Whales are of particular interest, for both toothed and baleen species, although not herbivorous, ingest large amounts of insoluble polysaccharide in the chitin skeletons of their cephalopod and crustacean prey, and have

voluminous and compartmentalized stomachs that are well adapted to bacterial fermentation of this material.

Further evidence of the role of SCFA in the digestion of plant fibre by herbivores was provided by the Cambridge school of animal physiology (Elsden *et al.*, 1946), which showed high levels of SCFA in the rumen of cattle, sheep and deer; these ruminants showed a second SCFA peak in the large intestine, corresponding to the peak shown by hindgut fermenters such as the horse, rabbit, rat and pig (Fig. 1.1). The carnivorous dog, despite its relatively poorly developed large intestine, also showed a peak of SCFA in that organ (Phillipson, 1947), presumably derived from microbial breakdown of protein; our own later observation of SCFA in the lumen of the cleansed and defunctioned human colon (Rubinstein, Howard & Wrong, 1969) suggests that endogenous mucoprotein is a contributor to this SCFA. Of interest was the finding that SCFA concentrations in the large intestine were similar in the various mammals studied, regardless of feeding habits or the existence of a foregut fermentation chamber, suggesting that the capacity of the large intestine, and sequestration of contents in the viscus, are more important than the composition of contents in determining the amount of SCFA produced.

The human gut

In humans, early studies on intestinal SCFA were confined to observations on faecal composition, initially by Brieger (1878) in Berne, later by several other European workers (Hecht, 1910; Schmidt & Strasburger, 1910; Bahrdt & McLean, 1914; Roux & Goiffon, 1921; Cecchini, 1923), who separated the individual components of SCFA after steam-distillation by differential precipitation of their metallic salts. Their recorded concentrations of total SCFA agreed well with more recent estimates, except for occasional improbably high values in infants' stools, possibly the result of continuing fermentation after the stool was passed. These early contributors recognized that SCFA arose from microbial fermentation, but speculated little on the nature of the substrate involved, or on any further role of SCFA in nutrition or intestinal function, other than to suggest that it might be important in fermentative diarrhoea. The 1929 articles from the St Louis group were a landmark; these investigators reviewed earlier work, improved the old methods, and studied the factors influencing faecal SCFA in normal humans (Olmsted *et al.*, 1929; Grove, Olmsted & Koenig, 1929). Three normal subjects had mean SCFA concentrations in their stools of 93–219 ml 0.1N acid/100 g of whole stool. In separate articles they later described the 'disappearance' (i.e. fermentation)

of various fibre fractions during alimentary transit; in three subjects taking several forms of fibre supplement, disappearance of cellulose was 0–72%, and of 'hemicellulose' was 32–89% (Williams & Olmsted, 1935, 1936). Their methods of establishing faecal recovery of these forms of fibre were imperfect by modern standards, but these workers were the first to study the digestibility of these fibre fractions in humans, and to suggest that they were a major source of faecal SCFA.

My personal involvement with SCFA in the human large intestine was originally accidental, and arose in the sixties from my dissatisfaction, as a physician interested in the salt and water composition of body fluids, that very little was known about the ionic composition of human faeces, although on average 75% of their weight is water. Human stool had long been submitted to analysis after incineration or various forms of digestion by strong acids, but the information obtained gave no information about organic constituents, or indicated which faecal constituents are precipitated in an insoluble form or exist in solution in faecal water. Microscopic examination of stool showed that this faecal water must exist in two main forms: (1) a discontinuous intracellular phase sequestered in the numerous inclusion bodies (chiefly bacteria, but also pips and seeds, protozoal bodies and desquamated epithelial cells) that are present in faeces, and (2) a continuous extracellular phase, in which these inclusion bodies are suspended and which itself is in contact with the intestinal mucosa until faeces are expelled from the body. An analogy with the intracellular and extracellular compartments of total body water is obvious. However, it seems that gastroenterologists have not conceived of faecal water as composed of these compartments, for the few other attempts that have been made to analyse the water of healthy human faeces have treated it as a single compartment (Tarlow & Thom, 1974; Bjork, Soergel & Wood, 1976).

A further difficult problem in the analysis of faecal water arises from the fact that within the large intestine the material is alive, containing many millions of bacteria, most of which are obligatory anaerobes. The metabolism of these bacteria continues after a stool has been passed, but many die and autolyse when they are exposed to the hostile aerobic environment outside the body. An indication of the continuing effect of bacterial activity on the composition of stool outside the body was provided by the early demonstration that the material continues to evolve ammonia (Gamble, 1915). Exploring this phenomenon, we found that ammonia was generated under all conditions of faecal incubation, but increased more when conditions were unfavourable to bacterial survival (Vince *et al.*, 1976); its generation could not be prevented even by complete sterilization of faeces with gamma-irradiation, and in this

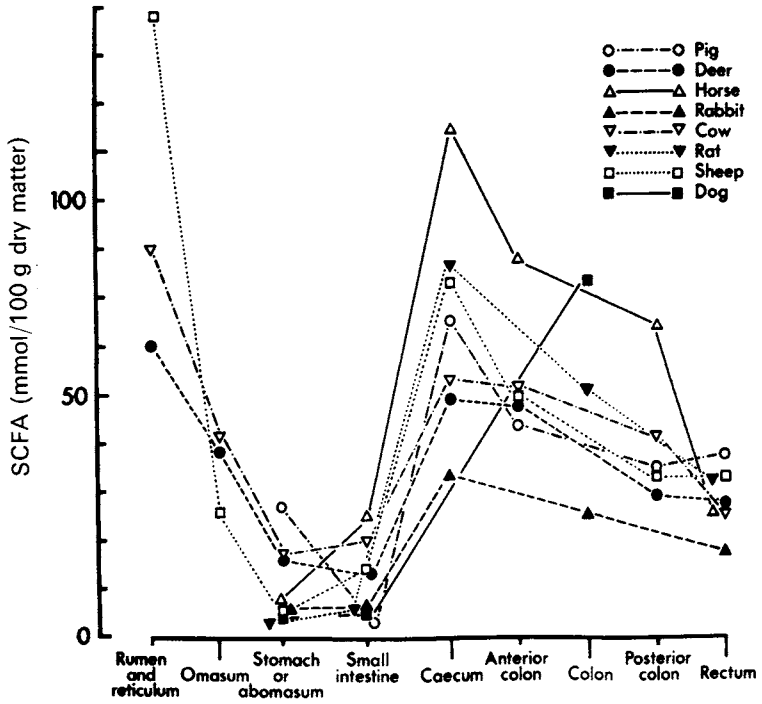

Fig. 1.1. SCFA concentrations at various levels in the digestive tract of different mammals. Redrawn from Elsden *et al.* (1946) and Phillipson (1947). From Wrong *et al.* (1981) with permission of the publishers.

circumstance we concluded that it was the result of deamination of bacterial proteins caused by residual bacterial enzymes. This study also showed that faeces incubated outside the body generated SCFA, whether or not conditions were favourable to bacterial survival. This practical problem of faecal generation of SCFA outside the body, despite efforts being made to bring these chemical processes to a stop, had previously been noted by Olmsted *et al.* (1929); history repeats itself, for it has recently been rediscovered, by a distinguished gastroenterology research unit, that generation of SCFA and osmoles by human stool continues even when specimens are kept on ice (Hammer *et al.*, 1989).

A less important problem has been the unit of measurement of intestinal concentrations. In the early literature these were variously expressed as per weight of water content (g/100 g), per weight of total contents, or per weight of solids (as in Fig. 1.1). The first of these is the most useful for readers, as it allows calculation of molal concentrations per litre of water; the other two units require assumptions regarding the proportion of intestinal solids, and simple adoption of a mean value of 25% (the average for human faeces)

Table 1.1. *Concentrations (mean values ± 1 SD) in stool water of human subjects, obtained by faecal dialysis* in vivo *(Wrong* et al., *1965; Rubinstein* et al., *1969), high-speed centrifugation (Tarlow & Thom, 1974) and ultrafiltration (Bjork* et al., *1976)*

	Dialysis *in vivo*	High-speed centrifugation	Ultrafiltration
mosmol/kg	376 ± 27	475 ± 66	410 ± 83
pH	7.0 ± 0.5	6.1 ± 0.7	—
Na (mmol/l)	32 ± 28	32 ± 19	24 ± 21
K (mmol/l)	75 ± 25	70 ± 33	83 ± 24
NH_4 (mmol/l)	14 ± 8	33	42 ± 11
Cl (mmol/l)	16 ± 8	23 ± 8	11 ± 4
Total CO_2 (mmol/l)	40 ± 15	7.3	18 ± 7
Organic anion (mmol/l)	172 ± 39	203 ± 27	196 ± 54

would produce errors with fluids of greatly different solid content. SCFA concentrations have usually been expressed as acetic-acid equivalents, or as millilitres of 0.1N mineral acid, from which it is easy to calculate SI units.

In vivo faecal dialysis

It was to get round these various problems that I and my colleagues introduced the technique of *in vivo* faecal dialysis, in which the contents of the large intestine were dialysed through small semipermeable cellulose capsules containing colloid, which were swallowed and later passed in the faeces. The contents of these dialysis capsules were removed for analysis as soon as a stool was passed, without further handling of stool samples (Wrong, Morrison & Hurst, 1961; Wrong *et al.*, 1965). Since the method was introduced, 32 years ago, we have recovered 5742 of these dialysis capsules from the faeces of 109 subjects, without clinical harm resulting, so the method is fairly safe, though we have deliberately avoided using it in infants or any subject whose past history suggests the possibility of an intestinal stricture. The fluid obtained by this technique can have equilibrated only with the extracellular component of faecal water, so it was to be expected that it should show some differences in composition from samples of total stool water obtained after faeces had been homogenized and either ultracentrifuged or ultrafiltered under aerobic conditions outside the body. However, the higher concentrations of organic anion and ammonia and higher osmolality (especially the last two), and the lower pH and concentration of bicarbonate of these samples of stool water, shown in Table 1.1, probably resulted mainly

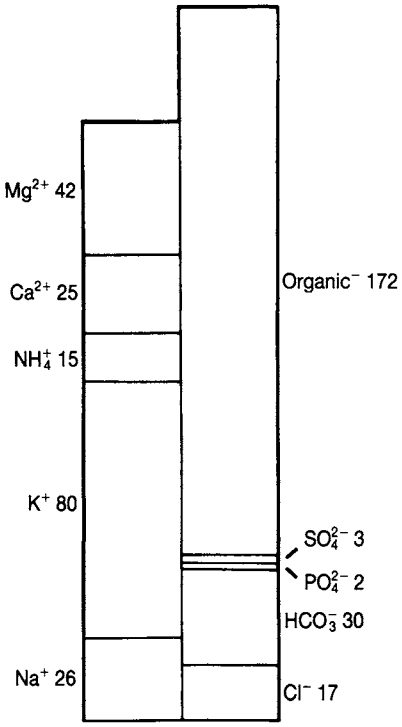

Fig. 1.2. Ionogram from faecal dialysate *in vivo* in humans. Mean values are shown, expressed in mequiv./l. From Wrong *et al.* (1981) with permission of the publishers.

from continued bacterial metabolism and autolysis outside the body, for the inorganic ion concentrations were very similar to those of the *in vivo* faecal dialysate, which would not be expected if they were samples of different compartments of faecal water.

The fluid produced by *in vivo* dialysis was usually clear, of a variable brown colour, with pH 7.0 $\pm$ 0.5 and osmolality 376 $\pm$ 27 mosmol/kg. This slight hypertonicity appeared to be the result of bacterial activity, for specimens became isotonic when intestinal bacterial activity was suppressed by oral use of insoluble broad-spectrum antibiotics (Wilson *et al.*, 1968). Ionic composition was very variable, mean values being shown as a Gamble ionogram (mequiv./l) in Fig. 1.2. To us the most surprising feature of the fluid was the vast gap between inorganic cations (potassium, sodium, ammonium, calcium and magnesium) and anions (chloride, bicarbonate, phosphate and sulphate), averaging 136 mequiv./l of missing anion. By exclusion, this gap could only be occupied by organic anion, and titration using a technique adapted from an old method for urine confirmed that it was indeed organic anion, with a

mean p*K* value of just under 5. (This method tended to overestimate organic anion, and this overestimation, and the error caused by averaging small numbers of determination, were probably the main factors giving rise to the apparent higher mean anion than cation concentration in Fig. 1.2, a difference that cannot exist, as it implies that human faeces carry a negative charge of some millions of volts!) Glancing at an alphabetical table of p*K* values of organic acids, we were impressed that acetic acid appeared near the top of the table with a p*K* of 4.8. Subsequent gas chromatography showed that acetate was indeed the main organic anion in this fluid, followed by propionate and *n*-butyrate, in average proportions of 59:22:19 (Rubinstein *et al.*, 1969), ratios which are similar to those seen in the rumen. We also observed that faecal dialysate pH and total organic anion concentration were strongly negatively correlated, suggesting that faecal pH was largely determined by intestinal generation of SCFA.

This was probably the first time that the contribution of SCFA to the ionic and osmolar composition of faecal water was determined in humans. Its contribution was much greater than we had ever suspected, largely because we were ignorant of earlier work measuring SCFA in human faeces, which by 1965 was largely forgotten, or the observations on other mammals. Some colleagues in my own salt-and-water field expressed doubts whether our high SCFA values were correct; my suspicion is that they had become so used to the concept that the ionic and osmolar structure of body fluids is predominantly inorganic (the salt of 'salt and water'), that they found it hard to accept that here was one body fluid of which the major ionic and osmolar component was organic. The veterinary physiologists I met were not at all surprised; why, they reasoned, should humans be different from other mammals? (see Fig. 1.1).

SCFA as an energy source in humans

Standard texts on food composition, such as *McCance and Widdowson's The Composition of Foods* (Paul & Southgate, 1978), make the assumptions that in humans starch and glycogen are completely digested, but that no other dietary polysaccharides have any value as energy sources. These two assumptions may seem reasonable in texts used for the design of diets, but are oversimplifications in view of recent work both on the indigestibility of a variable portion of dietary starch (Cummings & Englyst, 1991), and on the extent of fibre breakdown in the large intestine. Because of the variability of starch and fibre intake in human diets, it is unlikely that the two errors (which in energy terms have opposite effects) will often cancel themselves out.