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**THE USE OF BREATH HYDROGEN TESTING TO
EVALUATE CARBOHYDRATE
MALABSORPTION IN DOGS**

**A thesis presented in partial fulfilment of the requirements for the
degree of Master of Veterinary Science in Small Animal Medicine at
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Abstract

The use of the breath hydrogen test in this thesis has focussed primarily on the study of carbohydrate assimilation in healthy dogs and in dogs with gastrointestinal disease. The gastrointestinal handling of dietary carbohydrates and the rationale, applications, and limitations of the breath hydrogen test have been reviewed. Studies were undertaken to investigate the effect of food particle size on carbohydrate digestion and the effect of dehydration on breath hydrogen concentrations in healthy dogs. In addition, breath hydrogen testing was used to assess the assimilation of four commonly used commercial carbohydrate sources in dogs with gastrointestinal disease. In each study, expired breath samples were collected at regular intervals after the ingestion of a carbohydrate test meal. The excretion of hydrogen in the breath was compared between groups, mainly by analysis of the areas under the breath hydrogen concentration versus time curves.

The reduction of food particle size was found to cause starch granule disruption and significantly decreased the amount of malassimilated rice. The assimilation of corn, however, did not appear to be altered by reducing the food particle size. Instead, an earlier rise of breath hydrogen concentrations occurred, indicating more rapid orocolic transit and/or fermentability of the smaller corn particles.

Five percent dehydration, induced by food and water deprivation, was found to significantly elevate breath hydrogen concentrations and was associated with a significantly greater number of flatus-contaminated breath samples. This increased breath hydrogen excretion associated with increased flatulence in dehydrated dogs was attributed to a greater "net" production of hydrogen within the gastrointestinal tract.

Finally, breath hydrogen concentrations were not found to vary significantly between four different extrusion cooked carbohydrate sources (wheat, potato, corn, rice). However, it could not be concluded that these carbohydrates were assimilated to a similar extent as *in vitro* fermentation results revealed marked differences in the amount of hydrogen produced per gram of carbohydrate fermented at different fermentation times. Individual dogs with gastrointestinal disease appeared to differ in their ability to assimilate the four different carbohydrate sources. In addition, dogs with exocrine pancreatic insufficiency were found to malabsorb significantly more carbohydrate than the dogs with mild inflammatory bowel disease.

In conclusion, food processing to reduce carbohydrate particle size appears to increase the assimilation of rice. Methods to reduce the particle size of rice should be considered in the formulation of veterinary therapeutic diets for the management of diarrhoea. Mild dehydration

appears to increase breath hydrogen excretion. This suggests that breath hydrogen tests should not be performed on animals that are suboptimally hydrated until their hydration deficits have been restored. The marked individual variation of carbohydrate assimilation noted in dogs with gastrointestinal disease, suggests that clinicians should consider altering the carbohydrate source offered to dogs with intractable malassimilation, with the aim of finding the carbohydrate best tolerated. Finally, direct comparison of breath hydrogen concentrations should not be used to compare the digestibility of different complex carbohydrates unless an accurate means is available of comparing the amount of hydrogen produced per gram of substrate fermented *in vivo*.

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CHAPTER 1

A REVIEW OF GASTROINTESTINAL HANDLING OF DIETARY CARBOHYDRATES

Introduction

In both man and dogs, carbohydrates represent the principle energy source in the diet. Carbohydrates are estimated to account for approximately 50% of the daily caloric intake of humans, and a similar percentage of daily calories are provided by carbohydrates in commercial dog foods. Compared to proteins and fats, carbohydrates also represent the most economical energy source available (Berdanier 1995). Dietary carbohydrates are classified as sugars (monosaccharides and disaccharides), oligosaccharides, and polysaccharides (starch and fibre) (Table 1.1).

The digestion of the majority of carbohydrates in the small intestine is achieved by the action of pancreatic α -amylase and the intestinal brush border carbohydrases: lactase, sucrase-isomaltase and glucoamylase (Dahlqvist and Semenza 1985, Gray 1992). These enzymes work to degrade carbohydrates to their constituent monosaccharides, which are primarily absorbed across the small intestinal mucosa by active transport. Although carbohydrates are normally extensively digested and absorbed in the small bowel, the degree of assimilation is determined by a number of factors that relate to the form and source of the food, and the gastrointestinal function of the host (Dreher and Berry 1984, Cummings and Englyst 1995).

Carbohydrates that are indigestible, or that escape digestion and absorption in the small bowel, are usually further digested by the colonic microflora (Macfarlane and Cummings 1991). Fermentation of dietary carbohydrates within the large bowel results in the formation of short chain fatty acids (SCFA) and gas (primarily carbon dioxide, hydrogen, and methane). The formation of SCFAs provides the host with an important energy source and helps to maintain colonic health. Excessive carbohydrate malabsorption, however, overwhelms the ability of the large bowel to ferment and/or absorb fermentation end products, and causes a variety of clinical signs recognised as carbohydrate intolerance (Casparly 1986).

Carbohydrates in the Diet

Monosaccharides

Monosaccharides are the elementary units of all carbohydrates. Although carbohydrates were originally thought to be hydrates of carbon, this chemical definition was limiting, and was revised to include polyhydroxy aldehydes or ketones and their derivatives. A monosaccharide consists of a single polyhydroxyl aldehyde or ketone unit and has the empirical formula $(\text{CH}_2\text{O})_n$ where n is 3-9 (Berdanier 1995). The common dietary monosaccharides contain five or six carbon atoms (pentoses and hexoses). Glucose, fructose and galactose represent the most important dietary sugars, although very little glucose and virtually no galactose are ingested as monosaccharides. Glucose may be ingested as a free sugar in fruits (especially grapes), honey, and in vegetables, but it is mainly derived from the breakdown of oligosaccharides and starch. Fructose is the sweetest natural sugar, and is abundant in honey and in fruits, particularly in apples, oranges, pears and cherries. A number of sugar alcohols (sorbitol, xylitol) are also present in small amounts in some fruits and vegetables, and are produced industrially for use in slimming and diabetic diets (Kearsley and Sicard 1989, Guesry and Secretin 1991). Monosaccharides represent the smallest group of dietary carbohydrates in man, and are likely to be even less important in the majority of canine diets.

Oligosaccharides

Oligosaccharides consist of two to ten monosaccharide units joined by glycosidic linkages (Berdanier 1995). The principle dietary oligosaccharides are the disaccharides sucrose, maltose and lactose. Sucrose, is a disaccharide of glucose and fructose joined by an α -1,2 linkage. Unlike most of the other natural sugars, it is not a reducing sugar. It is produced industrially from sugar cane and sugar beet, and is also found in fresh fruit (eg., peaches, apricots and melons) and vegetables. It is the most frequently used commercial sweetener and represents approximately 30% of the total consumption of carbohydrates in man. Maltose contains two molecules of glucose joined by an α -1,4 link and occurs naturally in some fruits (eg., grapes), but is primarily derived from the hydrolysis of starch. Lactose is a disaccharide of glucose and galactose joined by a β -1,4 linkage. It is exclusive to the milk of most mammals and represents an important energy source in neonates. Other oligosaccharides of nutritional significance include stachyose and raffinose. These oligosaccharides are found mainly in legumes (particularly in soy), and are indigestible in the small bowel due to the absence of

digestive enzymes capable of their hydrolysis (Kearsley and Sicard 1989, Levin 1989, Guesry and Secretin 1991)

In comparison to people, naturally occurring disaccharides are unlikely to form a large component of the daily carbohydrate intake in adult dogs. The content of sucrose containing foods in the majority of canine diets is low, and recommendations to avoid milk consumption in dogs is common due to the high incidence of lactose intolerance in this species.

Starch

Starch is the major storage polysaccharide of plants and the principle dietary carbohydrate source of man and dogs. Starch consumption in humans is estimated to be approximately 60% of the total carbohydrate intake (Caspary 1986) and is likely to be even higher in most canine diets. Starch exists in two forms: amylose and amylopectin. Both of these macromolecules are referred to as homopolysaccharides because they are polymers of one monosaccharide (glucose).

Amylose constitutes 20-30% of most starches and is an essentially linear molecule in which the glucose units are linked by α -1,4 glycosidic bonds. Although it is described as a linear molecule, amylose occurs as a helical structure which readily forms complexes with inorganic ions (eg., iodine). The chains vary in molecular weight (200-2000 glucose residues) depending on the starch source, with an average weight of 100 kDa (600 glucose units). It is now thought that some branching α -1,6 linkages probably occur in the amylose chain.

Amylopectin is the major component of most starches, accounting for approximately 70-80%. Amylopectin is a highly branched structure containing repeating glucose units linked by both α -1,4 and α -1,6 bonds (Figure 1.1). The α -1,6 links represent approximately 5% of the linkages and determine the branching nature of the amylopectin chain. The amylopectin molecule is extremely large, varying between 10,000-100,000 glucose residues. Although the ratio of amylose to amylopectin in starch is generally 1:4, waxy starches and high amylose starches exist which contain almost pure amylopectin and >70% amylose respectively (Kearsley and Sicard 1989, Gray 1992, Annison and Topping 1994, Berdanier 1995).

Resistant starch

Resistant starch (RS) is a relatively new term that is defined as the sum of starch and starch degradation products not absorbed in the small intestine of healthy individuals (Asp 1994, Muir *et al* 1994). The four main forms of RS that have been identified, include: physically entrapped starch, ungelatinised B-type starch granules, retrograded amylose, and chemically modified starch (see "Carbohydrates in the large bowel" for further details).

Resistant starch is included in many nutritionist's definition of fibre, although current analysis methods do not recover all forms of RS. For example, common analytical methods will categorise physically entrapped and ungelatinised B-type RS as starch (provided sample disintegration and gelatinisation are sufficient), while retrograded amylose will be included in many fibre analyses.

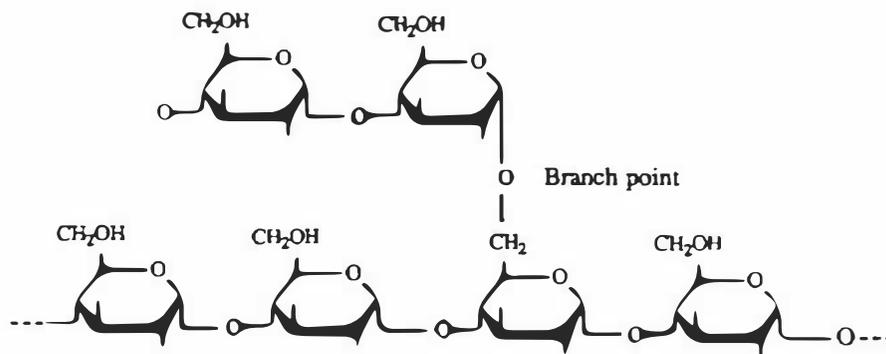


Figure 1.1 An example of the molecular structure and α -1,6 branching point of the amylopectin chain (adapted from Berdanier 1995)

Fibre (Non-starch polysaccharides)

The term “dietary fibre” was originally introduced to describe the indigestible cell wall material of plant foods, including: polysaccharides, lignin, and other compounds such as proteins, tannins and cutin that are not digested and absorbed in the small bowel. This definition was later redefined to include all indigestible polysaccharides and lignin, and is a widely accepted definition of fibre by many nutritionists today (Asp 1994, Sunvold 1996). However, a move to redefine fibre as non-starch polysaccharides (NSP) exists (Englyst and Cummings 1990). Non-starch polysaccharide is considered a preferable term to fibre, as it is precisely measurable and gives the best index of plant cell wall polysaccharides, which is in keeping with the original concept of dietary fibre.

The principle types of NSP in the diet are cellulose, hemicellulose, and pectin. Cellulose is the most abundant structural polysaccharide of plants and consists of a straight chain polymer of glucose residues joined by β -1,4 glycosidic linkages. Hemicellulose is unrelated to cellulose structurally and consists of a xylose polymer with β -1,4 links and side chains of arabinose and other sugars. Pectin is found principally in fruit and is a polymer of methyl D-galacturonate. Other NSPs in the diet include inulin, guar, seed mucilages and plant gums (Berdanier 1995, Macfarlane and Cummings 1991).

Fibres have traditionally been classified according to their solubility and viscosity characteristics, which helps to predict their physical properties. In general, soluble fibres (noncellulosic polysaccharides eg., hemicellulose, gums, pectins) contain a low lignin content, increase the viscosity of the intraluminal contents within the gastrointestinal tract, and are highly fermentable. Insoluble fibres (eg., cellulose) are generally less fermentable with greater water holding capacity which increases faecal bulk. However, many exceptions exist, as not all soluble fibres are highly fermentable, nor are all insoluble fibres poorly fermentable. It has been suggested that classifying fibres by their fermentability characteristics (ie., fermentable, non-fermentable) may be more appropriate, as this provides more direct evidence of a fibre's physiological effects within the lower gastrointestinal tract (Jenkins 1988, Wursch 1991, Reinhart and Sunvold 1996).

Table 1.1 Main food carbohydrates

Monosaccharides	Polysaccharides
Glucose	Starch
Fructose	Amylopectin
	Amylose
	Modified food starches
Disaccharides	Non-starch polysaccharides
Sucrose	Cellulose
Lactose	Hemicellulose
	Pectins
Oligosaccharides	β -glucans
α -Galactosides	Fructans eg, inulin
Raffinose, stachyose	Gums
Fructans	Mucilages
Fructooligosaccharides	Algal polysaccharides

Carbohydrates in the Small Bowel: Digestion and Absorption

Salivary Hydrolysis

Starch digestion begins in the mouth in species capable of producing salivary α -amylase. Salivary α -amylase is thought to be a recent evolutionary development, and among mammals it's production has only been observed in primates, rodents and lagomorphs (Meisler and Gumucio 1986). In these species, it's action is identical to that of pancreatic amylase, initiating the hydrolysis of amylose and amylopectin by cleavage of the internal α -1,4 links (see "Intraluminal digestion"). Salivary amylase is thought to play only a minor role in the digestion

of carbohydrates in humans, due to its rapid degradation in gastric acid and the high activity of pancreatic amylase. However, salivary amylase may receive considerable protection from acid degradation in the stomach when it is associated with starch or starch degradation products, and therefore may contribute significantly to intraluminal digestion. An important digestive role of salivary amylase has been suggested in infants, where only low levels of pancreatic amylase are present (Alphers 1987, Gray 1992).

Gastric Hydrolysis

Despite the lack of specific carbohydrate degrading enzymes in the stomach, carbohydrates may undergo gastric non-enzymatic (acid) hydrolysis (Alphers 1987). The extent of this hydrolysis is dependent on many factors, including gastric emptying time, carbohydrate concentration and the degree of starch granule dispersion.

Intraluminal Digestion

Pancreatic and salivary α -amylases are the only carbohydrases present in the duodenal luminal fluid. Pancreatic amylase is secreted from the pancreas in response to meal-stimulated secretin and pancreozymin release. It can be found both free in the duodenal lumen and attached to the digestive surface of the enterocyte. The concentration of pancreatic amylase is highest in the duodenum where rapid and efficient intraluminal starch hydrolysis normally occurs (Alphers 1987).

The specificity of pancreatic α -amylase is for the internal α -1,4 linkages of starch (Gray 1992). The catalytic subsites of α -amylase bind to five of the glucose residues of amylose and amylopectin adjacent to the terminal reducing glucose unit, and cleave between the second and third α -1,4 linked glucosyl residue. Alpha-amylase is unable to hydrolyse the α -1,6 branch linkages in amylopectin, or cleave the α -1,4 links adjacent to these branching points because of steric hindrance. In addition, α -amylase has less specificity for the smaller maltooligosaccharides which may bind to the enzyme but fail to span the scission site. In general, very little free glucose is released under physiological conditions. Therefore, the principle end products of amylose hydrolysis within the intestinal lumen are the disaccharide maltose and the trisaccharide maltotriose. In contrast to amylose degradation, the principle end products of amylopectin hydrolysis within the intestinal lumen includes branched oligosaccharides (α -limit dextrins consisting of one or more α -1,6 links and an average of 5-10 glucose residues), as well as maltose and maltotriose. Alpha-limit dextrins account for as much

as one third of amylopectin breakdown products. Figure 1.2 demonstrates the hydrolysis of amylose and amylopectin by pancreatic α -amylase

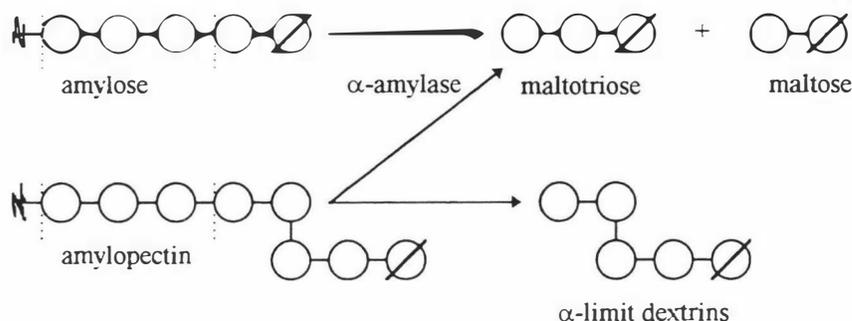


Figure 1.2 Alpha-amylase hydrolysis of amylose and amylopectin chains (adapted from Alpers 1987).

The activity of pancreatic amylase in newborn animals is generally low. Pancreatic amylase activity increases rapidly in puppies and kittens to adult levels after five weeks of age, while the activity in adult dogs varies according to the amount and digestibility of starch in the diet (Meyer and Kienzle 1991).

Intestinal Surface Digestion

It is necessary for carbohydrates to be completely hydrolysed to their respective monosaccharides before absorption across the enterocyte is possible. The enzymes responsible for the digestion of dietary disaccharides and the final digestion of the starch oligosaccharide breakdown products to their respective monosaccharides, are the brush border carbohydrases (surface oligosaccharidases, membrane bound glucosidases, brush border disaccharidases).

The brush border carbohydrases are large glycoproteins of the intestinal brush border membrane. They are originally synthesised as large protein precursors within the enterocyte, trimmed and glycosylated intracellularly and transferred to the brush border membrane where they are anchored by the terminal hydrophobic segment of their protein chains. The bulk of their total mass, including their catalytic sites, is located within the intestinal lumen. Many of the brush border carbohydrases are referred to as complexes, as they exist as a single large polypeptide prior to the splitting of this "pro-form" into two separate subunits by proteases (Alpers 1987, Dahlqvist and Semenza 1985, Gray 1992).

The integral brush border carbohydrases in mammals include the β -glucosidase; lactase-glycosylceramidase (lactase-phloridzin hydrolase), and the α -glucosidases; glucoamylase (maltase-glucoamylase, amyloglucosidase), sucrase-isomaltase (sucrase- α -

dextrinase) and trehalase (Dahlqvist and Semenza 1985, Alpers 1987, Galand 1989, Gray 1992). Nomenclature for these enzymes commonly varies and can be confusing. The term “maltase” is often used to refer to glucoamylase, sucrase-isomaltase or both of these enzymes, because maltase activity is associated with both subunits in both of these molecules.

Lactase is one of the subunits of the β -glycosidase complex. Unlike the sucrase-isomaltase and glucoamylase complexes, lactase-glycosylceramidase is cleaved into its two active subunits intracellularly. Lactase has a broad substrate specificity (lactose, cellobiose, cellotriose), however lactose is the only substrate of nutritional significance. The glycosylceramidase subunit splits phlorizin and a number of aryl- β -glycosides. Lactase hydrolyses lactose into its component monosaccharides; glucose and galactose. Lactase activities decrease in most mammals after weaning and unlike other brush border carbohydrases, lactase activity cannot be induced by feeding high levels of lactose or galactose (Casparly 1986). Lactase activity generally decreases to adult levels in dogs by 29-61 days of age (Welsh and Walker 1965). However, lactase activity may remain high until 16 weeks of age in some puppies (Meyer and Kienzle 1991).

The sucrase-isomaltase complex is cleaved extracellularly by pancreatic proteases after its insertion into the brush border membrane. The sucrase and isomaltase subunits remain noncovalently associated, but the physical interaction and the cleavage process is not thought to increase their activities. The sucrase subunit is specific for sucrose and maltooligosaccharides, while the isomaltase subunit is capable of hydrolysing both α -1,4 and α -1,6 linkages (although prefers the smaller α -dextrin molecules). Therefore, the isomaltase subunit displays: isomaltase activity (action against the α -1,6 links of small α -dextrins), α -limit dextrinase activity (action against the α -1,6 links of large α -dextrins) and maltase activity. In man, it is estimated that the sucrase-isomaltase complex accounts for 100% of sucrase activity, 80% of maltase activity, most of the isomaltase activity, and some of the α -limit dextrinase activity in the small intestine.

The glucoamylase complex is also cleaved extracellularly by pancreatic proteases into two subunits in most species, although some exceptions have been identified (eg., pigs, pigeons). The two subunits of glucoamylase have very similar or identical substrate specificities, and are occasionally referred to as “heat-stable maltases” due to the different heat stabilities of their protein domains. They are both responsible for the sequential removal of glucose residues from the non-reducing end of the α -1,4 chain. In addition, glucoamylase is also thought to possess significant α -limit dextrinase activity and some isomaltase activity. It is estimated that glucoamylase accounts for 20% of the maltase activity, most of the α -limit dextrinase activity, and a minor component of the isomaltase activity of the small intestinal mucosa.

The substrate specificity of trehalase is strictly limited to trehalose, a glucose disaccharide present in mushrooms, insects, moulds and yeasts. Trehalase has been detected in the intestinal mucosa of dogs, but appears to be absent in the intestine of cats (Hore and Messer 1968). It's importance in the digestive function of dogs is likely to be negligible, given that trehalose is an unusual component of canine diets.

The substrate specificities of most of the α -glucosidases overlap, so that these enzymes are able to work on the final hydrolysis of starch in a complimentary manner. Glucoamylase initiates the digestion of the branched oligosaccharides, removing individual glucose residues in a sequential manner. Isomaltase (and probably also glucoamylase) is essential for the cleavage of the α -1,6 branching link, and sucrase is the preferred enzyme for the final cleavage of the chain once it has been reduced to two or three glucose residues. Table 1.2 outlines the principle substrates, main enzyme activities/specificities, and final products of brush border carbohydrase digestion.

Table 1.2 Major intestinal brush border carbohydrases in the mammal

<i>Enzyme</i>	<i>Principle substrates</i>	<i>Main enzyme activity</i>	<i>Products</i>
Lactase	Lactose	<u>Lactase</u> : β -1,4 linkage	Glucose, galactose.
Glucoamylase	Starch degradation products: maltooligosaccharides and large α -Dextrins.	<u>Maltase</u> : α -1,4 linkage <u>α-limit dextrinase</u> : α -1,6 linkage of penta or hexasaccharides.	Glucose, residual oligosaccharides.
Sucrase	Sucrose, small maltooligosaccharides (maltose, maltotriose).	<u>Sucrase</u> : α -1,2 linkage <u>Maltase</u> : α -1,4 linkage	Glucose, fructose.
Isomaltase	α -Dextrins (preferably small), maltooligosaccharides.	<u>Isomaltase</u> : α -1,6 linkage of tri- or tetrasaccharides. <u>Maltase</u> : α -1,4 linkage	Glucose
Trehalase	Trehalose	<u>Trehalase</u> : α -1,1 linkage	Glucose

The activity of the brush border carbohydrases in humans rises in the duodenum, peaks in the distal jejunum, and then declines to low levels in the ileum (Alpers 1987). There appears to be a similar pattern in dogs, as brush border enzyme activity has been reported to be highest in the proximal and middle sections of the small bowel (Welsh and Walker 1965). The digestion of carbohydrates is normally extremely efficient in man (complete by the mid-jejunum), and is thought to exceed the efficiency of the intestine to absorb monosaccharides. Therefore, monosaccharide absorption (rather than digestion) is considered the rate limiting step of starch assimilation in humans. However, this is not the case for lactose assimilation, where the rate limiting step is considered to be the hydrolysis of lactose by lactase.

The activities of carbohydrases in the brush border vary with age, diet and disease. Brush border carbohydrase activities in healthy adult dogs are reported to be highest for maltase, followed by sucrase, glucoamylase, lactase and cellobiase respectively (Noon *et al* 1977). While lactase levels decrease in dogs post weaning, the activities of maltase and sucrase increase with age (Welsh and Walker 1965, Meyer and Kienzle 1991). This presumably occurs in response to feeding carbohydrates. Adaptation of brush border enzymes (increase in enzyme amount or activity) to feeding specific carbohydrates or carbohydrate-rich diets, may also occur in adult animals (Levine 1989). In man, a high dietary intake of sucrose or fructose (but not glucose) increases maltase and sucrase activities. Feeding high starch diets to rats has been shown to cause more efficient monosaccharide absorption, thereby increasing brush border carbohydrase activity by the reduction of end product inhibition. Increased maltase and sucrase activities in response to a high starch diet have also been demonstrated in adult dogs (Meyer and Kienzle 1991). The increased carbohydrase activity that occurs with feeding carbohydrates is a result of more rapid enzyme synthesis and slower enzyme degradation. The adaptive period over which this occurs has been shown to be as short as 12-21 hours in rats fed sucrose (Riby and Kretchmer 1984). Regulation of brush border carbohydrases in humans occurs by end product inhibition, and degradation by pancreatic and bacterial proteases (Alpers 1987). End product inhibition is thought to be important in limiting monosaccharide levels in the intestinal lumen and thereby controlling the osmotic load. The degradation of brush border carbohydrases by proteases causes a decline in carbohydrase activity after feeding, and explains the increase in enzyme activity seen with exocrine pancreatic insufficiency in man and in dogs. Prolonged fasting is also associated with decreased enzyme activity in humans.

Monosaccharide Transport

The three main monosaccharides that are well absorbed across the enterocyte in humans are glucose, galactose and fructose. Other monosaccharides are usually present in the intestinal

lumen in insignificant amounts (Caspary 1986). Given that sucrose and lactose are only present in small quantities in most canine diets, glucose absorption is by far the most important absorptive process in the dog.

Absorption of sugars across the enterocyte into mucosal blood involves: (i) sugar movement to the mucosal surface from the intestinal bulk phase and unstirred water layer, (ii) movement across the brush border membrane, and (iii) movement across the basolateral membrane (Levin 1994). Movement of oligosaccharides and monosaccharides across the bulk phase and unstirred water layer occurs by passive diffusion. The unstirred water layer is a layer of fluid that lies adjacent to the intestinal lining and mixes poorly with the bulk phase of the luminal fluid. It consists of a series of water laminae that extend outward from the intestinal mucosa and becomes progressively more stirred as it blends with the bulk phase (Dietschy 1971). The thickness of this water layer may limit mono- and oligosaccharide access to the brush border surface, and therefore delay sugar and starch hydrolysis and absorption.

The movement of monosaccharides across the brush border membrane may occur by active transport, facilitated diffusion, and passive diffusion. Glucose and galactose (which have nearly identical structures) compete for the same carrier mechanism; the Na^+ -glucose cotransporter (SGLT1). This transporter has been successfully cloned and sequenced from rabbit intestine by Hediger *et al* (1987). It transfers hexoses against a concentration gradient and is Na^+ dependent. Glucose or galactose and intraluminal sodium are bound to separate sites on the transport protein at a ratio of two Na^+ ions to a single hexose. The hexose and Na^+ are transported intracellularly along the steep Na^+ electrochemical gradient between the lumen (100-140 mM) and intestinal epithelial cell (50 mM). Once the hexose and Na^+ move to the interior surface of the membrane, their affinity for the transport protein is decreased and they are released into the cytosol. The driving force for the active hexose transport is provided by the Na^+/K^+ ATPase pump in the basolateral membrane. This pump maintains the Na^+ electrochemical gradient by moving Na^+ extracellularly. Active transport systems for hexoses have also been reported in the small intestine and colon of dogs and the small intestine of cats (Scharrer and Wolffram 1987).

Facilitated diffusion of hexoses are thought to occur when intraluminal concentrations are high, and to involve SGLT1 as well as a separate glucose transporter known as GLUT 5. In comparison to facilitated and active transport, the contribution of passive diffusion to the movement of hexoses across the enterocyte is small. Once intracellular, the hexoses may exit the enterocyte by three routes: a small amount undergoes reverse transportation back into the intestinal lumen via the brush border transport protein, but the majority transverse the basolateral membrane by facilitated diffusion by a Na^+ -independent "serosal" carrier system

Carbohydrates in the Large Bowel: Fermentation

It was originally thought that carbohydrate assimilation in the small bowel was so efficient that only the indigestible NSPs reached the colon in appreciable amounts. Since the 1980's however, human studies utilising ileostomy models, ileal intubation, and breath hydrogen testing have identified starch as a major substrate entering the colon and undergoing fermentation (Levitt 1983, Cummings and Englyst 1991). The "carbohydrate gap", a term used to describe the increase in faecal bulk in humans eating mixed diets, that could not be explained by the NSP content alone, can be explained by the physiological malabsorption of starch. Starch that escapes digestion and absorption in the small intestine and passes into the colon has been termed "resistant starch" (RS).

Carbohydrates that are not assimilated in the small bowel and enter the colon in significant amounts in man include: RS, NSPs, and a small amount of simple carbohydrates (eg., sugars and oligosaccharides). Resistant starch is considered to be the most important of these carbohydrates in quantitative terms, and is estimated to account for 10% of all starch in western diets. In dogs, it is likely that RS and NSP constitute the bulk of the carbohydrates reaching the large bowel because sugars are not usually fed in significant amounts. Other important substrates metabolised in the colon include mucins (glycoproteins) secreted by the colonic epithelium, and protein and nitrogen sources derived from the diet and endogenous secretions (Cummings and Englyst 1987, Cummings and MacFarlane 1991).

Resistant Starch

Resistant starch can be categorised into four main groups of starch which resist enzymatic digestion in the small bowel (Macfarlane and Cummings 1991, Muir *et al* 1994).

1. *Starch physically inaccessible to digestion.* Food form is an important factor in the digestibility of starch, with whole or partially milled grains and seeds, and larger food particle size (decreased surface-to-volume ratio) frequently acting as a barrier to starch digestion. Starches present in such foods may be considered to be physically trapped, with intact cell walls preventing complete swelling and dispersion of the starch granules, and larger particles limiting amylase access to the amylose and amylopectin chains (Snow and O'Dea 1981, Wursh *et al* 1986, Heaton *et al* 1988). In addition, the porosity of starch substrates may also limit enzyme accessibility (Colonna *et al* 1992).

2. *Raw (ungelatinised) starch granules of x-ray diffraction pattern type B and C.* In plants, starches are present as granules which contain randomly orientated crystalline regions in

an amorphous matrix. The crystalline regions represent amylose and amylopectin chains in a double helical conformation. The crystalline structure of the starch within the granule can be of x-ray diffraction pattern A, B, or C (intermediate between type A and B rather than a distinct crystalline structure) as described by Katz (1934). In general, type A is present in cereal starches, type B in tuber starches and amylose-rich starches, and type C in legume starches. The type of x-ray diffraction pattern is thought to be largely influenced by the length of the amylopectin chains, which are shorter in type A starches. The nature of the packing of the helices, and the amount of associated water, within the starch granule also differs between type A & B starches. The crystalline nature of the starch dictates its susceptibility to enzymatic digestion. Type A starches (eg, oats) can be digested ungelatinised, whereas type B starches (eg, potato) and type C starches (eg, peas) are poorly digestible until they are gelatinised. Gelatinisation of the starch granule refers to the breakdown of the crystalline regions due to the application of heat and moisture. This process results in swelling and disruption of the starch granule, rendering amylose and amylopectin more accessible to enzymatic hydrolysis (Gallant *et al* 1992, Annison and Topping 1994).

3. *Retrograded starch.* Retrograded starch is formed when gelatinised starch is stored (as with processed foods). On cooling, drying or freezing, the amylose chains (and amylopectin to a lesser degree) in gelatinised starches reassociate and slowly recrystallise with time. The type B x-ray diffraction pattern, which is highly resistant to enzymatic digestion, is mostly formed. The type A x-ray diffraction pattern may also occur if the starch is held at high temperatures for extended periods after gelatinisation. Retrogradation of starch can be increased by repeated heating and cooling cycles, and retrograded amylose is considered to be more resistant to digestion than retrograded amylopectin. Therefore, highly digestible cooked starches may retrograde with storage and become more resistant to digestion. The temperature of the starch on cooling, moisture content, time, and starch source all affect the degree of retrogradation that occurs (Gallant *et al* 1992, Annison and Topping 1994).

4. *Chemically modified starch.* This type of starch has only recently been included in the RS definition, and includes starch which has been modified (eg., cross-linking, substitution) for use in the food industry, to improve various characteristics of food products. Unlike the other three types of RS, this starch does not occur naturally (Muir *et al* 1994).

Many other factors also affect the digestibility of starch and influence the amount of carbohydrate reaching the colon. These factors include the formation of complexes with other food components such as fat or protein, and the presence of NSPs or amylase inhibitors. The gastrointestinal transit time of the food, extent of food chewing and many other gastrointestinal variables can also affect the extent of starch assimilation (see Chapter 3 for more details).

Non-starch polysaccharides

Quantitatively, the NSPs represent the second most important group of carbohydrates that enter the colon in humans. The common dietary NSPs are cellulose, hemicellulose, pectins, and a range of chemically related substances (eg., inulin, guar, seed mucilages and plant gums). Given that NSPs are not digested in the small bowel to any significant extent, it is reasonable to assume that all NSPs in the diet enter the large intestine. However, not all NSPs undergo bacterial fermentation in the colon. The extent of fermentation of a NSP is influenced by a number of factors including the NSPs water solubility, extent of cell wall lignification, and the presence of silica, cuticular substance and tannins. The gastrointestinal transit time is also an important determinant of the extent of NSP fermentation. In man, it is estimated that at least 50% of cellulose and 80% of non-cellulosic polysaccharides are digested by colonic microorganisms (Cummings and Macfarlane 1991).

Sugars and Oligosaccharides

Lactose, raffinose and stachyose are the principle sugars and oligosaccharides that escape small bowel digestion in humans. Raffinose and stachyose are α -galactosides which are found in small percentages in legumes, but are unable to be assimilated in the small bowel of mammals because the α -galactosidase required for their digestion is not present. Sugar alcohols (eg., sorbitol and xylitol) are only slowly digested and absorbed, and are therefore also commonly malabsorbed. Small amounts of oligosaccharides, such as the indigestible fructooligosaccharides found in vegetables such as artichokes, onions and some root crops, also reach the colon (Wursh 1991, Cummings and Macfarlane 1991). As for humans, malabsorbed oligosaccharides are only likely to account for a small percentage of the carbohydrates reaching the colon in dogs. However, the use of soy products in commercial dog foods may provide significant quantities of α -galactosides, and lactose malabsorption is likely to occur in dogs fed generous quantities of milk.

Colonic Fermentation of Carbohydrates

Fermentation in the colon refers to the anaerobic breakdown of dietary and endogenous substrates by the bacterial populations present. The large intestine is an extremely complex ecosystem which is estimated to contain over 400 different bacterial species in man, with faecal bacterial counts ranging from 10^{11} - 10^{12} bacteria/gram dry weight. More than 95% of these bacterial species are anaerobes (Cummings and Macfarlane 1991). Dogs and cats have both been shown to contain very active colonic bacteria and possess moderate fermentation capabilities (Reinhart and Sunvold 1996). The fermentation of substrates in the colon is an

ATP-generating process, whereby the colonic flora obtain energy for growth and maintenance of cellular function. Dietary carbohydrates represent the most important substrate for microbial metabolism in the colon. Other important substrates for colonic fermentation include mucins secreted by colonic goblet cells and protein and nitrogen sources. Mucins are a particularly important substrate in the absence of food intake, as they provide fuel for the colonic microorganisms during periods of starvation (Cummings and Macfarlane 1991).

A range of different nutritional types of bacteria exist in the colon, reflecting the variety of substrates available for their growth. These bacteria include the saccharolytic (polysaccharide degrading) species which hydrolyse complex carbohydrates, bacteria which degrade protein, peptides and amino acids, and methanogens and other bacteria which feed on the intermediate products of fermentation. An overview of carbohydrate degradation and fermentation in the large bowel is shown in Figure 1.4.

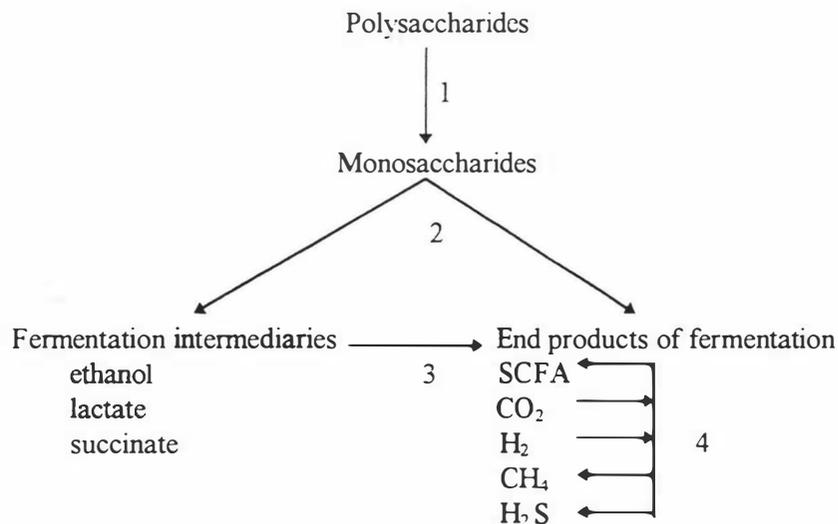


Figure 1.4 Overview of carbohydrate degradation and fermentation in the large bowel. (1) Hydrolysis of polysaccharides by bacterial polysaccharidases and glycosidases; (2) fermentation of sugar monomers; (3) bacterial fermentation of intermediate products; (4) hydrogen disposal by methanogenesis, sulphate reduction and acetogenesis

The principle polysaccharide degrading bacteria are probably gram negative anaerobes belonging to the genus *Bacteriodes* (Macfarlane and Cummings 1991). These bacteria are able to synthesise a wide range of cell-associated polysaccharide depolymerases and glycosidases, enabling them to grow on a variety of polysaccharides. Other anaerobic bacteria involved in the breakdown of polysaccharides in man include the genera *Bifidobacterium*, *Ruminococcus*, *Eubacterium*, *Lactobacillus* and *Clostridium*. Interestingly, there is evidence that *Bifidobacterium* species may not be commonly present in canine colonic flora (Willard 1996). It

is likely that enzymes from many different bacterial species participate in the degradation of complex carbohydrates. Some bacteria are capable of producing a wide range of polysaccharidases and glycosidases which enables them to digest their substrates extensively, while other bacterial species are only capable of hydrolysing smaller polysaccharide fragments, and are thus reliant on the presence of other polysaccharide degrading bacteria for their growth. This process is referred to as “crossfeeding of carbohydrates” (Macfarlane and Cummings 1991). Polysaccharide degradation is considered to be the rate limiting step in the conversion of carbohydrates to SCFAs (Cummings and Englyst 1987). However, recent observations have suggested that the conversion of monosaccharides to SCFAs may be more limiting (Hammer *et al* 1990).

Sugar monomers produced from the hydrolysis of polysaccharides are fermented by the majority of intestinal anaerobes via the Embden-Meyerhof-Parnas pathway to pyruvate (Miller and Wolin 1979). The significant products of pyruvate metabolism are: the SCFAs acetate, propionate and butyrate; the gases hydrogen (H_2) and carbon dioxide (CO_2); intermediate products such as succinate, lactate and ethanol; and energy utilised by the bacteria. The intermediate products lactate, succinate and ethanol are electron sink products formed by bacteria to dispose of excess reducing power during fermentation. They do not usually accumulate to any extent under normal conditions, as they are further metabolised (i.e., to propionate) by bacteria capable of utilising these products (Macfarlane and Cummings 1991).

Hydrogen, like lactate, succinate and ethanol is also an electron sink product that is produced by many bacterial species. Bacteria which produce significant amounts of lactate, succinate or ethanol as electron sink products do not produce much H_2 . Hydrogen is both an important intermediate and end product of fermentation. Several types of H_2 utilising bacteria exist and play an important role in maintaining efficient fermentation by disposal of H_2 . This is necessary as high partial pressures of H_2 inhibit the reoxidation of reduced pyridine nucleotides; a coenzyme essential for bacterial fermentation of sugars. Hydrogen metabolism also shifts fermentation to a more oxidised end product resulting in a higher energy yield, which is advantageous for both the host and colonic microflora. The main disposal routes of H_2 occur via methanogenesis, acetogenesis, and dissimilatory sulphate reduction (Gibson *et al* 1990, Macfarlane and Cummings 1991, Christl *et al* 1992).

Methanogenesis occurs when significant numbers of methanogenic bacteria are present in the colon and use H_2 to reduce either CO_2 or methanol to methane (CH_4). Between 24-65% of adult humans have the ability to produce CH_4 in their breath as well as H_2 , indicating that significant methanogenesis is occurring in the colon (Corazza *et al* 1994). The ability of humans to produce CH_4 is thought to vary between ethnic groups and have a strong familial association

(Bond *et al* 1971, Pitt *et al* 1980). The predominant methanogenic species is *Methanobrevibacter smithii* which uses H_2 to reduce CO_2 to CH_4 . *Methanosphaera stadtmaniae* is less common and uses H_2 to reduce methanol to CH_4 . Methanogenic bacteria are very efficient H_2 scavengers: CH_4 producing faeces consume H_2 much more rapidly than non- CH_4 producing faeces (Strocchi and Levitt 1992a, Strocchi *et al* 1993b), and methanogens are estimated to be capable of reducing total H_2 excretion by 75% (Christl *et al* 1992). Methanogens are the predominant H_2 consumers in man, and are thought to reside mainly in the left colon where the pH is slightly higher than the proximal colon and more favourable for their growth (Flourie *et al* 1990). Methane production in dogs is probably less important than in humans. Studies in dogs have failed to detect the presence of CH_4 in gases aspirated from intestinal segments and have also failed to isolate methanogens from the faeces (Richards and Steggerdia 1966, Miller and Wolin 1986). However, CH_4 has been identified in gas produced from *in vitro* fermentation of canine ileal chyme with soya oil meal (Grubler *et al* 1987), and may therefore play at least a minor role in H_2 disposal in this species. Given that excessive volumes of intestinal gas would occur without significant H_2 disposal, it is likely that alternative pathways of H_2 disposal also occurs in dogs.

Sulphate reducing bacteria (SRB) use H_2 to convert sulphate to hydrogen sulphide (H_2S). The activity of SRB depends on the availability of sulphate. People who are not capable of excreting CH_4 are more likely to have faecal flora that display increased rates of sulphate reduction and increased concentrations of sulphide. The increased prevalence of sulphate reducing action in non- CH_4 producing individuals is thought to be due to SRB outcompeting the methanogenic bacteria for H_2 . The optimal pH for dissimilatory reduction of sulphate appears to be more alkaline (7.5) than the optimal pH of methanogenesis (7.0) (Gibson *et al* 1990, Strocchi *et al* 1993b).

A third potential mechanism for H_2 uptake in fermentation is acetogenesis by reduction of CO_2 to acetate. This is an important process in the gut of termites, and has been shown to occur in human faecal slurries (where CH_4 production was absent or low) and in non-methanogenic and methanogenic rats (Lajoie *et al* 1988). It appears that acetogenesis may be a more important means of H_2 disposal in non-methanogenic individuals and in acidic environments (optimal pH 6.0). The incubation of human faecal samples has demonstrated that a major fraction of H_2 catabolism could not be accounted for by methanogenesis or sulphate reduction, and may be due to acetogenesis or another unreported pathway of H_2 catabolism (Strocchi *et al* 1993b).

Many factors have been found to influence the fermentation reactions of intestinal bacteria, and therefore will influence the type of intermediate and end products produced. These

factors include: the amount and composition of the substrate present (starch produces high levels of butyrate in comparison to some NSPs, sugars produce more H_2 than polysaccharides), the rate of substrate depolymerisation, the numbers, specificities, preferences and fermentation strategies of individual gut species, and the availability of inorganic terminal electron acceptors. Finally, extensive microbial fermentation in the hindgut requires an adequate environment for microbe growth, primarily an adequate fluid medium and sufficient neutralising power (eg., high bicarbonate concentrations in ileal outflow) to maintain the pH in a favourable range for fermentation and SCFA absorption (Macfarlane and Macfarlane 1993, Rechkemmer *et al* 1988).

The fate and physiological significance of fermentation end products

Many of the important effects of carbohydrate metabolism in the large bowel can be attributed to the production and rapid absorption of SCFAs. The production of SCFAs results in an estimated energy salvage of 62% of fermentable carbohydrate entering the large bowel, contributes to salt and water homeostasis in the colon, helps regulate gastrointestinal motility and plays an integral role in the maintenance of colonic epithelial cell health (Macfarlane and Cummings 1991). Short chain fatty acid production also inhibits the colonisation of pathogenic bacteria by reducing colonic pH and by encouraging the growth of indigenous bacterial populations.

The predominant SCFAs produced in the colon are acetate, propionate and butyrate. These SCFAs account for 85-95% of the total SCFA production in humans, with estimated molar ratios of 57:22:21 respectively (Macfarlane and Cummings 1991). However, the proportions of SCFAs produced varies widely with the type of fermentable substrate entering the large intestine (Reinhart and Sunvold 1996). Short chain fatty acid concentrations are highest in the proximal colon where the supply of fermentable substrates is large.

The absorption of SCFAs is rapid, and it is estimated that 95% of the SCFAs generated are absorbed. Short chain fatty acid absorption is concentration dependent, associated with bicarbonate accumulation in the colon, and stimulates the absorption of salt and water (Rechkemmer *et al* 1988, Remesy *et al* 1989). The absorption of SCFAs primarily occurs in the non-ionised (protonated) form by passive diffusion, although some facilitated transfer is probably involved. In the dog, SCFA and sodium are absorbed at the same rate, and is sufficient to account for all the osmotic absorption of H_2O from the colon (Herschel *et al* 1981).

Short chain fatty acids are metabolised by colonocytes, providing the colonic epithelium with their major source of energy. Butyrate appears to be the preferred respiratory fuel for colonocytes in humans and has been found to exert regulatory effects on nucleic acid

metabolism, thereby promoting normal cell phenotype by stabilisation of DNA and repair of damage. Therefore, butyrate appears to be an important substrate for normal colonic epithelial nutrition, growth and differentiation, and is thought to play an important role in the prevention of colon cancer (Annison and Topping 1994).

Once absorbed into the portal vein, SCFAs pass into the liver where significant hepatic clearance occurs. Acetate is the only SCFA found in significant amounts in peripheral blood, due to the preferential uptake of butyrate by colonocytes and the efficient clearance of proprionate by the liver. Acetate is a valuable tissue fuel in man, and is mainly metabolised by cardiac, skeletal muscle and the brain. Proprionate is the only SCFA which can effect net conversion to glucose, and while an important gluconeogenic precursor in ruminants, it is unknown if the role of proprionate is similar in monogastrics. Up to 30% of the total energy requirement of some nonruminant species may be met by SCFA production and metabolism, with species containing large and complex hindguts extracting the greatest amount of energy from this process (Cummings and Macfarlane 1991, Sunvold 1996).

The other major end products of fermentation in the colon are the gases H_2 and CO_2 . Methane may also be produced in significant amounts in some people (see above). Colonic gas is eliminated by a number of routes. Hydrogen may act as an intermediate product of fermentation and be further metabolised by colonic flora as discussed above, enter the portal circulation and be excreted by the lungs, or be expelled as flatus. The movement of gases across the gastrointestinal tract has been shown to follow the physical laws of diffusion, and is thus dependent on the partial pressures differences between the gastrointestinal lumen and circulation, and the characteristics of the membranes these gases must cross (McIver *et al* 1926). Because H_2 and CH_4 are foreign to the metabolism of the body, and are therefore normally present in the circulation in negligible amounts, their production in the colon is followed by rapid diffusion into the blood and lungs. The proportion of H_2 and CH_4 expired in breath has been shown to vary with total excretion rates (Christl *et al* 1992). At low total H_2 excretion rates of up to 200 ml/d as much as 65% may be expired in the breath, while greater total excretion rates are associated with lower proportions of H_2 excreted in the breath (eg., 25%). Methane excretion was found to follow a similar pattern. The physiological significance of gas production is unknown.

Carbohydrate Intolerance

The terms carbohydrate malabsorption and carbohydrate intolerance are not synonymous. Carbohydrate malabsorption refers to ingested carbohydrates which are not fully assimilated in the small bowel, thereby entering the digestive system of the large bowel. Carbohydrate intolerance, however, refers to the symptoms of abdominal discomfort, borborygmus, flatulence and diarrhoea which may result from carbohydrate malabsorption. In normal individuals, carbohydrate malabsorption is a physiological phenomenon important to the health and maintenance of normal colonic function, and is a means of energy generation for the host. Whether or not carbohydrate malabsorption results in carbohydrate intolerance depends on a number of variables. Some of these variables include: the amount and type of carbohydrate being malabsorbed, the rate at which the malabsorbed carbohydrate reaches the colon (i.e., the influence of gastric emptying and small bowel transit time), the response of the small intestine to the osmotic load, the metabolic capacity of the colonic flora, the buffering and absorptive capacity of the colon, and the sensitivity of the general visceral afferent system to intestinal distension (Ravich and Bayless 1983, Caspary 1986)

The bacterial fermentation of unabsorbed carbohydrates may affect stool weight by three separate mechanisms. An increase in solute load from the conversion of sugars to SCFA (1 mol disaccharide results in 3.7 mol SCFA) and the retention of cations associated with SCFA formation, will increase stool weight. Alternatively, a reduction in solute load due to the rapid absorption of SCFA across the colonic mucosa may decrease stool weight. Although diarrhoea from carbohydrate malabsorption was originally believed to be due to the formation of SCFAs and their subsequent osmotic effect in the colonic lumen, it has been shown that diarrhoea can be markedly attenuated by the production and absorption of SCFAs (Hammer *et al* 1989). Unfermented carbohydrates, however, exert an osmotic drive in the lumen, and increased faecal carbohydrates correlate closely with diarrhoea when carbohydrate intake is high and malabsorption present (Holtug *et al* 1992). Therefore, the overall result of carbohydrate malabsorption on faecal weight depends on the amount of unfermented carbohydrate and the net effect of SCFA formation and absorption. Minor impairment of carbohydrate assimilation does not result in changes in stool volume and pH due to the complete fermentation of carbohydrate in the colon. The salvage of energy from the malabsorbed carbohydrate under these conditions is likely to be maximal. The malabsorption of carbohydrates in greater quantities may cause a saturation of the fermentation system or conditions unfavourable for fermentation (e.g., rapid transit rate or decrease in pH below five). Under such conditions, unfermented carbohydrates,

unabsorbed SCFAs, and retained cations result in diarrhoea, decreased faecal pH, and intact sugars appearing in the faeces (Hammer *et al* 1989, Hammer *et al* 1990, Holtug *et al* 1992). Abdominal discomfort and borborygmus commonly occurs with carbohydrate malabsorption due to bowel distension and increased peristalsis from the osmotic effect of the carbohydrates in the small intestine. Flatulence results from excess intestinal gas which is produced during fermentation (Ravich and Bayless 1983).

Carbohydrate malabsorption and intolerance may result from an interruption in any of the normal steps involved in carbohydrate degradation and absorption. Impaired intraluminal digestion due to a loss of pancreatic amylase, results in carbohydrate malabsorption in man and dogs with exocrine pancreatic insufficiency (Kerlin *et al* 1984, Ladas *et al* 1993, Washabau *et al* 1986b). Decreased pancreatic enzyme release (eg., lack of cholecystokinin and secretin production with severe small intestinal mucosal disease), or conditions unsuitable for optimal pancreatic enzyme action (eg., acidic duodenum from accelerated gastric emptying or lack of pancreatic or biliary bicarbonate secretion), may also result in impaired intraluminal digestion (Williams 1996).

Impaired brush border membrane digestion and monosaccharide absorption represents another potential defect in the gastrointestinal handling of carbohydrate. Primary disaccharidase deficiencies are well documented in man, including: lactase deficiency, sucrase-isomaltase deficiency, trehalase deficiency, and glucoamylase deficiency which has only recently been identified (Saavedra and Perman 1989, Gray *et al* 1976, Bergoz 1971, Lebenthal *et al* 1994). Glucose-galactose malabsorption is a primary brush border abnormality that occurs in humans due to a defect in the Na⁺/glucose cotransporter (Turk *et al* 1991). In dogs, the only documented primary brush border abnormality is lactase deficiency (Hill 1972). However, secondary deficiencies of brush border enzymes and transport proteins commonly occur with many gastrointestinal diseases in both man and dogs. Small intestinal disease results in carbohydrate malabsorption because of decreased enterocyte numbers (morphological damage eg., villus atrophy), and/or due to decreased enterocyte function (microvillus damage without any obvious change in villous architecture) (Batt 1980). Examples of small bowel diseases that have been documented, or are likely to result in secondary disaccharidase deficiencies in dogs, include: acute infectious enteritis, parasite infestation, and chronic mucosal diseases such as inflammatory bowel disease, lymphangiectasia, gastrointestinal neoplasia, and small intestinal bacterial overgrowth (Williams 1996). Other causes of secondary carbohydrate malabsorption reported in humans include: food sensitivities, gastrointestinal surgery, protein-calorie malnutrition, drugs (antibiotics, cytotoxic agents), and immunodeficiency syndromes (eg., HIV) (Ushijima *et al* 1995). Malabsorption of lactose is the most prominent example of secondary

carbohydrate malabsorption in humans. This is thought to occur because lactase activity is the rate limiting step in the digestion of lactose (in contrast to the digestion of other carbohydrates where monosaccharide absorption is considered to be the major factor limiting their assimilation). In addition, the superficial location of lactase on the brush border membrane probably makes this enzyme more sensitive to gastrointestinal insults (Caspary 1986).

References

- Alpers DH: Digestion and absorption of carbohydrates and proteins. In: Johnson LR (ed) *Physiology of the Gastrointestinal Tract*. Raven Press, New York, 1987. pp1469-1487.
- Annisson G, Topping DL: Nutritional role of resistant starch: chemical structure vs physiological function. *Annu Rev Nutr* 14:297-320, 1994.
- Asp N-G: Nutritional classification and analysis of food carbohydrates. *Am J Clin Nutr* 59:679S-681S, 1994.
- Batt RM: The molecular basis of malabsorption. *J Small Anim Pract* 21:555-569, 1980.
- Berdanier CD: *Advanced Nutrition: Macronutrients*. CRC Press, Boca Raton, 1995. pp 159-208.
- Bergoz R: Trehalose malabsorption causing intolerance to mushrooms. *Gastroenterology* 61:909, 1971.
- Bond JH, Engel RR, Levitt MD: Factors influencing pulmonary methane excretion in man. *J Exp Med* 133:572-588, 1971.
- Caspary WF: Diarrhea associated with carbohydrate malabsorption. *Clin Gastro* 15:631-655, 1986.
- Christl SU, Murgatroyd PR, Gibson GR, Cummings JH: Production, metabolism, and excretion of hydrogen in the large intestine. *Gastroenterology* 102:1269-1277, 1992.
- Colonna P, Leloup V, Buleon A: Limiting factors of starch hydrolysis. *Eur J Clin Nutr* 46:S17-S32, 1992.
- Corazza GR, Benati G, Strocchi A, *et al*: The possible role of breath methane measurement in detecting carbohydrate malabsorption. *J Lab Clin Med* 124:695-700, 1994.
- Cummings JH, Englyst HN: Fermentation in the human large intestine and the available substrates. *Am J Clin Nutr* 45:1243-1255, 1987.
- Cummings JH, Englyst HN: Measurement of starch fermentation in the human large intestine. *Can J Physiol Pharmacol* 69:121-129, 1991.
- Cummings JH, Macfarlane GT: The control and consequences of bacterial fermentation in the human colon. *J Appl Bact* 70:443-459, 1991.

- Cummings JH, Englyst HN: Gastrointestinal effects of food carbohydrate. *Am J Clin Nutr* 61:938S-945S, 1995.
- Dahlquist A, Semenza G: Disaccharidases of small-intestinal mucosa. *J Pediatr Gastroenterol Nutr* 4:857-867, 1985.
- Dietschy JM, Sallee VL, Wilson FA: Unstirred water layers and absorption across the intestinal mucosa. *Gastroenterology* 61:932-934, 1971.
- Dreher ML, Dreher CJ, Berry JW: Starch digestibility of foods: a nutritional perspective. *Crit Rev Food Sci Nutr* 20:47-71, 1984.
- Englyst HN, Cummings JH: Non-starch polysaccharides (dietary fibre) and resistant starch. *Adv Exp Med Biol* 270:205-225, 1990.
- Flourie B, Etanchaud F, Florent C, *et al*: Comparative study of hydrogen and methane production in the human colon using caecal and faecal homogenates. *Gut* 31:684-685, 1990.
- Fujisawa T, Riby J, Kretchmer N: Intestinal absorption of fructose in the rat. *Gastroenterology* 101:360-367, 1991.
- Galand G: Brush border membrane sucrase-isomaltase, maltase-glucoamylase and trehalase in mammals. *Comp Biochem Physiol* 94B:1-11, 1989.
- Gallant DJ, Bouchet B, Buleon A, Perez S: Physical characteristics of starch granules and susceptibility to enzymatic degradation. *Eur J Clin Nutr* 46:S3-S16, 1992.
- Gibson GR, Cummings JH, Macfarlane GT, *et al*: Alternative pathways for hydrogen disposal during fermentation in the human colon. *Gut* 31:679-683, 1990.
- Gray GM, Conklin KA, Townley RRW: Sucrase-isomaltase deficiency: absence of an inactive enzyme variant. *N Eng J Med* 294:750-753, 1976.
- Gray GM: Starch digestion and absorption in nonruminants. *J Nutr* 122:172-177, 1992.
- Grubler B, Ingwersen M, Meyer H, Schunemann C: *In vitro* investigations on gas formation from ileum and colon chyme in dogs. *Nutrition, Malnutrition and Dietetics in the Dog and Cat*. 34-36, 1990
- Guesry PR, Secretin M: Sugars and nonnutritive sweeteners. In: Gracey M, Kretchmer N, Rossi E (eds) *Sugars in Nutrition*. Raven Press, New York, 1991. pp 33-53.
- Hammer HF, Santa Ana CA, Schiller R, Fordtran JS: Studies of osmotic diarrhea induced in normal subjects by ingestion of polyethylene glycol and lactulose. *J Clin Invest* 84:1056-1062, 1989.
- Heaton KW, Marcus SN, Emmett PM, Bolton CH. Particle size of wheat, maize, and oat test meals: effects on plasma glucose and insulin responses and on rate of starch digestion *in vitro*. *Am J Clin Nutr* 47:675-682, 1988.
- Hediger MA, Coady MJ, Ikeda TS, Wright EM: Expression cloning and cDNA sequencing of the Na⁺/glucose co-transporter. *Nature* 330:379-381, 1987.

- Herschei DA, Argenzio RA, Southworth M, Stevens CE: Absorption of volatile fatty acid, Na, and H₂O by the colon of the dog. *Am J Vet Res* 42:118-1124, 1981.
- Hill FWG: Malabsorption syndrome in the dog: a study of thirty-eight cases. *J Small Anim Pract* 13:575-594, 1972.
- Holtug K., Clausen MR, Hove H, Christiansen J, Mortensen PB: The colon in carbohydrate malabsorption: short-chain fatty acids, pH, and osmotic diarrhea. *Scand J Gastroenterol* 27:545-552, 1992.
- Hore P, Messer M: Studies on disaccharidase activities of the small intestine of the domestic cat and other carnivorous mammals. *Comp Biochem Physiol* 24:717-725, 1968.
- Jenkins DJA: Carbohydrates. In: Shils ME, Young VR (eds) *Modern Nutrition in Health and Disease*, 7th ed. Lea & Febiger, Philadelphia, 1988. pp 52-71.
- Katz JR: X-ray investigation of gelatinization and retrogradation of starch and its importance for bread research. *Bakers Weekly* 81:34-37, 1934.
- Kearsley MW, Sicard PJ: The chemistry of starches and sugars present in food. In: Dobbing J (ed) *Dietary Starches and Sugars in Man: A Comparison*, Springer-Verlag, London, 1989. pp 1-34.
- Kerlin P, Wong L, Harris B, Capra S: Rice flour, breath hydrogen, and malabsorption. *Gastroenterology* 87:578-585, 1984.
- Ladas SD, Giorgiotis K, Raptis SA: Complex carbohydrate malabsorption in exocrine pancreatic insufficiency. *Gut* 34:984-987, 1993.
- Lajoie SF, Bank S, Miller TL, Wolin MJ: Acetate production from hydrogen and [¹³C] carbon dioxide by the microflora of human faeces. *Appl Envir Micro* 54:2723-2727, 1988.
- Lebenthal E, Khin-Maung-U, Zheng B, *et al*: Small intestinal glucoamylase deficiency and starch malabsorption: a newly recognised alpha-glucosidase deficiency in children. *J Pediatr* 124:541-546, 1994.
- Levin RJ: Dietary carbohydrate and the kinetics of intestinal functions in relation to hexose absorption. In: Dobbing J (ed) *Dietary Starches and Sugars in Man: A Comparison*. Springer-Verlag, London, 1989. pp 89-111.
- Levin RJ: Digestion and absorption of carbohydrates-from molecules and membranes to humans. *Am J Clin Nutr* 59:690S-698S, 1994.
- Levitt MD: Malabsorption of starch: a normal phenomenon. *Gastroenterology* 85:769-770, 1983.
- Macfarlane GT, Cummings JH: The colonic flora, fermentation, and large bowel digestive function. In: Phillips SF, Pemberton JH, Shorter RG (eds) *The Large Intestine: Physiology, Pathophysiology, and Disease*. Raven Press, New York, 1991. pp 51-92.
- Macfarlane GT, Macfarlane S: Factors affecting fermentation reactions in the large bowel. *Proc Nutr Soc* 52:367-373, 1993.

- McIver MA, Redfield AC, Benedict EB. Gaseous exchange between the blood and the lumen of the stomach and intestines. *Am J Physiol* 76:92-111, 1926.
- Meisler MH, Gumucio DL: Salivary amylase: evolution and tissue-specific expression. In: Desnuelle P, Sjoström H, Noren O (eds) *Molecular and Cellular Basics of Digestion*, Elsevier Science Publishers B.V, 1986. pp 457-466.
- Meyer H, Kienzle E: Dietary protein and carbohydrates: relationship to clinical disease. *Purina International nutrition symposium in association with Eastern States Veterinary Conference. Orlando, Florida*. 1991, pp 13-26.
- Miller TL, Wolin MJ: Fermentations by saccharolytic intestinal bacteria. *Am J Clin Nutr* 32:164-172, 1979.
- Miller TL, Wolin MJ: Methanogens in human and animal intestinal tracts. *Syst Appl Microbiol* 7:223-229, 1986.
- Muir JG, Young GP, O'Dea K: Resistant starch- implications for health. *Proc Nutr Soc Aust* 18:23-32, 1994.
- Noon KF, Rogul M, Brendle JJ, Keefe TJ: Detection and definition of canine intestinal carbohydrases, using a standardized method. *Am J Vet Res* 38:1063-1067, 1977.
- Pitt P, de Bruijn KM, Beeching MF, *et al*: Studies on breath methane: the effect of ethnic origins and lactulose. *Gut* 21:951-959, 1980.
- Ravich WJ, Bayless TM: Carbohydrate absorption and malabsorption. *Clin Gastro* 12:335-356, 1983.
- Rechkemmer G, Ronnay K, Engelhardt W: Fermentation of polysaccharides and absorption of short chain fatty acids in the mammalian hindgut. *Comp Biochem Physiol* 90A:563-568, 1988.
- Reinhart GA, Sunvold GD: *In vitro* fermentation as a predictor of fibre utilization. In: Carey DP, Norton SA, Bolser SM (eds) *Recent Advances in Canine and Feline Nutritional Research*. Orange Frazer Press, Wilimington, Ohio, 1996. pp 15-24.
- Remesy C, Demigne C, Morand C: Metabolism and utilisation of short chain fatty acids produced by colonic fermentation. In: Schweizer TF, Edwards CA (eds) *Dietary Fibre- A Component of Food*. Springer-Verlag, London, 1989. pp 137-150.
- Riby JE, Kretchmer N: Effect of dietary sucrose on synthesis and degradation of intestinal sucrase. *Am J Physiol* 246:G757-763, 1984.
- Richards EA, Steggerdia FR: Production and inhibition of gas in various regions in the intestine of the dog. *Proc Soc Exp Biol Med* 122:573-576, 1966.
- Saavedra JM, Perman JA: Current concepts in lactose malabsorption and intolerance. *Annu Rev Nutr* 9:475-502, 1989.
- Scharrer E, Wolfram S: Absorption of nutrients in carnivorous animals. *Nutrition, Malnutrition and Dietetics in the Dog and Cat*. 6-10, 1990.

- Snow P, O'Dea K: Factors affecting the rate of hydrolysis of starch in food. *Am J Clin Nutr* 34:2721-2727, 1981.
- Strocchi A, Levitt MD: Factors affecting hydrogen production and consumption by human fecal flora: the critical roles of hydrogen tension and methanogenesis. *J Clin Invest* 89:1304-1311, 1992.
- Strocchi A, Ellis CJ, Levitt MD: Use of metabolic inhibitors to study H₂ consumption by human faeces: evidence for a pathway other than methanogenesis and sulfate reduction. *J Lab Clin Med* 121:320-327, 1993.
- Sunvold GD: Dietary fibre for dogs and cats: an historical perspective. In: Carey DP, Norton SA, Bolser SM (eds) *Recent Advances in Canine and Feline Nutritional Research*. Orange Frazer Press, Wilimington, Ohio, 1996. pp 3-14.
- Turk E, Zabel B, Mundlos S: Glucose/galactose malabsorption caused by a defect in the Na⁺/glucose cotransporter. *Nature* 350:354-356, 1991.
- Ushijima K, Riby JE, Kretchmer N: Carbohydrate malabsorption. *Pediatr Clin N Am* 42:899-912, 1995.
- Washabau RJ, Strombeck DR, Buffington CA, Harrold D: Use of pulmonary hydrogen gas excretion to detect carbohydrate malabsorption in dogs. *J Am Vet Med Assoc* 189:674-679, 1986.
- Welsh JD, Walker A: Intestinal disaccharide and alkaline phosphatase activity in the dog. *Proc Soc Exp Biol Med* 120:525-527, 1965.
- Willard MD: Effects of dietary fructooligosaccharide supplementation on canine small intestinal bacterial populations. In: Carey DP, Norton SA, Bolser SM (eds) *Recent Advances in Canine and Feline Nutritional Research*. Orange Frazer Press, Wilimington, Ohio, 1996. pp 15-24.
- Williams DA, Guilford WG: Malabsorption, small intestinal bacterial overgrowth, and protein-losing enteropathy. In: Guilford WG, Center SA, Strombeck DR, Williams DA, Meyer DJ (eds) *Strombeck's Small Animal Gastroenterology*. 3rd ed. W.B. Saunders, Philadelphia, 1996. pp 367-380.
- Wursh P, Del Vedovo S, Koellreutter B: Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *Am J Clin Nutr* 43:25-29, 1986.
- Wursh P. Dietary fibre and unabsorbed carbohydrates. In: Gracey M, Kretchmer N, Rossi E (eds) *Sugars in Nutrition*. Raven Press, New York, 1991. pp 153-168.

CHAPTER 2

A REVIEW OF THE BREATH HYDROGEN TEST

Introduction

Hydrogen gas (H_2), a by-product of microbial fermentation, can be measured in expired air and used as an indirect test for carbohydrate malabsorption. Fermentable substrates which reach the large bowel are rapidly metabolised by colonic bacteria, and a proportion of the H_2 produced diffuses into the portal circulation and is subsequently excreted in the breath. Simple techniques developed in man for the collection of expired breath and measurement of its H_2 content have also been applied to dogs (Washabau *et al* 1986a, Papsouliotis *et al* 1993b, Ludlow *et al* 1994b), cats (Muir *et al* 1991, Schlesinger *et al* 1993, Papsouliotis *et al* 1994), calves (Holland *et al* 1986, Naylor and Liebel 1995), and horses (Bracher *et al* 1995). The development of the breath H_2 test has provided an alternative diagnostic and investigative tool for several different areas in gastroenterology and carbohydrate nutrition. Since its development, the use of the breath H_2 test has involved many assumptions, some that have been shown to be correct and others that have been challenged. The following is a review of the rationale and applications of the breath H_2 test, and a discussion of its advantages and limitations.

Breath Hydrogen as a Measure of Intestinal Gas Formation

Hydrogen production and excretion

Hydrogen gas was identified in human flatus for the first time over 130 years ago (Ruge 1862). Hydrogen, along with methane (CH_4) and carbon dioxide (CO_2), were assumed to be produced endogenously rather than as a result of aerophagia, because of their extremely low concentrations in room air in contrast to their high concentrations in flatus. Intestinal bacteria were subsequently considered to be the source of H_2 and CH_4 production, because neither of these gases are produced by cellular metabolism in man, and negligible production was demonstrated in germ free rats and newborn infants (Levitt *et al* 1968, Levitt and Donaldson 1970). Furthermore, it was clearly demonstrated that H_2 evolved from gastrointestinal microorganisms in ileal and colonic dejecta when incubated anaerobically with a variety of

substrates (Calloway *et al* 1966). In this study, carbohydrate substrates were found to produce the largest amounts of H₂.

The major site of H₂ production within the gastrointestinal system corresponds to the area where the largest numbers of microorganisms reside; the large bowel. Using constant intestinal gas perfusion techniques, Levitt (1969) established that more than 99% of H₂ produced in the fasting state, and after the infusion of lactose into the gastrointestinal tract, was confined to the colon. Although only very low or undetectable concentrations of H₂ were present in the small bowel of fasting subjects, a small rise in H₂ production did occur in the small intestine following lactose infusion and the ingestion of beans (Levitt 1969). Richards and Steggerda (1966) added navy bean substrates to surgically prepared intestinal segments in dogs, and also demonstrated that the primary site of H₂ production in the canine bowel was the colon. However, they showed that small but significant volumes of gas were also produced in the duodenum and jejunum (approximately 15% the amount produced in the colon), and that relatively large amounts of gas may be produced in the ileum (up to 50% of the amount produced in the colon). Zentek *et al* (1993) have recently questioned the primary location of intestinal H₂ production and absorption relative to its excretion in the breath in dogs. Using dogs with duodenal and ileal fistulas, they showed that oral or duodenal, but not ileal, application of antibiotics reduced breath H₂ concentrations, and concluded that the majority of H₂ excreted in the breath of dogs is derived from the small intestine. However, despite demonstrating similar breath H₂ profiles between normal and fistulated dogs, the effect of the fistulas on small intestinal bacterial populations was not studied and may have contributed to increased ileal fermentation. Oropharyngeal microbes are also capable of fermenting substrates to produce H₂. When this occurs, an early breath H₂ rise is seen after feeding (usually within the first 30 minutes). This early rise can be attenuated by a 1% chlorhexidine mouth wash prior to meal ingestion. It has been proposed that oropharyngeal H₂ may contaminate exhaled H₂ in one of two ways: by direct contamination during exhalation, or by mucosal absorption of H₂ and subsequent excretion in the breath (Read *et al* 1985, Thompson *et al* 1985, Thompson *et al* 1986).

Disposal of intestinal H₂ occurs by three different routes: H₂ may be excreted in the breath and flatus, or further metabolised by the intestinal flora (Strocchi and Levitt 1992b). Due to the low concentrations of H₂ in tissues and air, H₂ readily diffuses along concentration gradients into the portal circulation and out into the atmosphere via the pulmonary circulation (McIver *et al* 1926). Hickey *et al* (1972) measured H₂ in the breath and the flatus, and demonstrated that approximately 50-100% of intestinal H₂ was exhaled in breath. Levitt (1969) estimated approximately 14% of intestinal H₂ to be exhaled in breath using a constant intestinal

gas perfusion technique, although it was recognised that this technique would underestimate pulmonary H₂ excretion by decreasing the intestinal partial pressures of H₂. By determining the rate of pulmonary versus rectal H₂ excretion, Levitt subsequently estimated the percentage of H₂ excreted via the lungs to be around 21%. He also concluded that breath H₂ accurately reflected intestinal H₂ production, as he found the proportion of intestinal H₂ excreted in the breath to be relatively constant for different subjects and different production rates (a good correlation of breath H₂ excretion and intestinal H₂ production was shown; $r = 0.94$). The amount and rate of H₂ excreted by the lungs has also been estimated by administering pure H₂ into the colon via a colonoscope, and measuring breath H₂ excretion over the following 30 minutes (Bjornekleit and Jenssen 1980). Total breath H₂ excretion calculated over the 30 minute period was found to be 2 and 1.5% of the instilled 100 and 500 ml volumes of H₂ respectively. These estimated proportions of H₂ excreted in the breath appear to be extremely low, and are probably a result of the short time period over which the breath samples were collected. More recently, Christl *et al* (1992) used a whole body calorimeter to measure total H₂ excretion. These workers found that breath H₂ excretion varied dramatically with varying rates of production. Overall, 58% of H₂ gas was excreted in the breath, with 65% excreted at low production rates (<200 ml/d), and as little as 25% at high production rates (>500 ml/d). Similar results were found in a subsequent study where breath H₂ excretion and flatus H₂ excretion were compared (Hammer 1993). Increasing volumes of H₂ excreted in the breath correlated positively with the H₂ volume in the flatus, but as total flatus volumes increased the percentage of H₂ excreted in the breath decreased (eg., colonic absorption of H₂ and excretion in the breath was estimated to be 90% at low flatus volumes and only 20% when flatus volume was high). Decreased colonic absorption of H₂ presumably occurs at higher gas production rates due to the more rapid gastrointestinal transit of gases, which decreases the time available for H₂ absorption.

H₂ consumption by intestinal microbes plays an important role in the disposal of intestinal H₂ and the determination of the "net" amount of H₂ available for excretion in the breath and flatus (Strocchi and Levitt 1992a & b) (see Chapter 1 for more information).

It is often assumed that H₂ production occurs with the fermentation of carbohydrate substrates only. However, there is sufficient evidence to suggest that proteins also are fermented by bacteria with subsequent H₂ production. Generally, the fermentation of proteins results in much less H₂ evolution than carbohydrates (Calloway *et al* 1966, Perman and Modler 1982, Carter *et al* 1981, Thompson *et al* 1986), although it has been demonstrated that very significant amounts of H₂ may be produced after feeding raw offal to dogs (Zentek *et al* 1993). Different microbial species and metabolic pathways are likely to be involved with the colonic degradation of proteins in comparison to carbohydrates.

Common endogenous and exogenous substrates that result in significant breath H₂ production include malabsorbed dietary sugars eg., lactose, fructose, sucrose (Levitt and Donaldson 1970, Metz *et al* 1975, Metz *et al* 1976b, Ravich *et al* 1983), indigestible carbohydrates eg., the legume oligosaccharides raffinose and stachyose (Calloway and Murphy 1968), carbohydrates that were once considered to be completely assimilated in the small bowel eg., starch of oats, corn and potatoes (Levine and Levitt 1981), fibre sources (McBurney and Thompson 1989) and carbohydrate rich glycoproteins eg., mucin (Perman and Modler 1982).

Breath collection techniques and sample storage

Several techniques have been developed for the collection of breath samples since the breath H₂ test was first introduced. These techniques include those that measure the total amount of H₂ exhaled in the breath over a given time period (total collection procedures, rebreathing techniques) (Levitt 1969, Bond and Levitt 1972, Newcomer *et al* 1975), and those that estimate total breath H₂ production via multiple samples of whole or end-tidal expiratory breath fractions collected at regular intervals (Metz *et al* 1976a, Solomons and Viteri 1978, Perman *et al* 1978). Despite the more precise and quantitative nature of total collection procedures (such as the closed, continuous, rebreathing system used by Bond and Levitt (1972) where the patient's head is enclosed in a polyvinyl hood), these methods are complicated and poor patient acceptability makes these techniques less useful for clinical purposes. For these reasons, interval sampling methods are more commonly used. End-expiratory sampling is the most frequently used interval sampling technique in man, as it is considered to approximate alveolar H₂ concentrations. Metz *et al* (1976a) first described this method utilising a Haldane-priestly tube, and showed that H₂ concentrations measured by this method correlated significantly with H₂ production measured by the rebreathing technique or by a total collection procedure ($r = 0.92$). However, because this method relies on patient cooperation for accurate end-expiratory sample collection, other techniques such as the nasal prong technique (Perman *et al* 1978), and the collection of total (mixed) expired air via an anaesthetic face mask (Solomons *et al* 1977) were developed for use in young children.

Due to the lack of patient cooperation encountered in veterinary medicine, breath collection techniques in animals have mainly been interval sampling of mixed expired air collected by face masks (Washabau *et al* 1986a, Holland *et al* 1986, Muir *et al* 1991, Pappasoulotis *et al* 1995). This technique involves the application of a close-fitting anaesthetic face mask, which is attached to a 1-4 litre capacity latex bag via a one-way, non-rebreathing valve. Samples are transferred directly from the bag to gas-tight syringes via a three-way stop cock, which is attached to the bag base. End-expiratory samples have also been collected from

dogs (Zentek *et al* 1993), and Muir *et al* (1991) has validated the use of an open-flow (semi closed) collection system for use in cats. The open-flow system is extremely well tolerated, and breath H_2 excretion determined from this system has been shown to correlate well with breath H_2 concentrations in expired air samples ($r = 0.94$). The relationship between the H_2 fraction/concentration (ppm) in expired breath has also been compared with H_2 excretion (ml/min) in dogs (Ludlow *et al* 1994b). A strong correlation ($r = 0.94$) was demonstrated, indicating that interval sampling of the breath H_2 fraction is a valid method of estimating total breath H_2 excretion.

One major criticism of mixed, expired breath collection is the dilution of H_2 by dead space (anatomical dead space and dead space in the collection apparatus). Anatomical dead space during tidal breathing is estimated to be around 30% in human paediatric patients, and dead space in the collection apparatus has been estimated to be below 4% if gas volumes are collected in excess of 3 litres, and the collection system receives a brief flushing period of expired breath prior to gas collection (Solomons *et al* 1977). Therefore, mixed air samples will represent approximately 70% of alveolar concentrations. In dogs, however, dilution of mask collected breath H_2 samples with atmospheric air has been estimated to be around 50-60% (Ludlow *et al* 1995). Interestingly, Holland *et al* (1986 & 1989) compared breath H_2 concentrations from mixed expired air and end-expiratory samples collected by tracheostomy in calves, and found the H_2 concentrations by both collection methods to be highly correlated ($r = 0.81$). They also found that both breath collection methods accurately predicted carbohydrate malabsorption after feeding milk to calves with chloramphenicol induced villus atrophy, and to calves inoculated with cryptosporidium. It was concluded that the dilution of expired H_2 by dead space is not a major factor in obtaining reliable results.

Special instructions on breathing techniques are given to human patients during breath collections in an attempt to reduce variations in breath H_2 concentrations due to changes in ventilation rates (Strocchi *et al* 1991). Increased minute ventilation (hyperventilation) has been shown to decrease breath H_2 concentrations, presumably due to a wash-out effect of alveolar H_2 (Perman *et al* 1985). Hyperventilation explains the decreased breath H_2 concentrations seen after exercise (Payne *et al* 1983), and also explains why hypoventilation is thought to increase breath H_2 concentrations during and immediately after sleep (Solomons and Viteri 1976, Metz and Jenkins 1977) (see "Variables and limitations of breath H_2 measurements" for more details). Unfortunately, controlled breathing is not possible during breath collections in animals, and therefore change in minute ventilation has the potential to cause error in breath H_2 determinations in veterinary patients.

Storage of breath samples is possible if the sample containers are gas tight. Sealed plastic 60 ml and 35 ml syringes stored at room temperature have been shown to leak H₂ at about 4-5% per 24 hours (Ellis *et al* 1988, Robb and Davidson 1981, Rosado and Solomons 1983). The gas in plastic syringes is thought to slowly diffuse through the plastic, while much higher gas losses occur in glass syringes due to leakage around the syringe barrel. A reduction in gas leakage to less than 1% per day can be achieved with cold storage at -20 °C (Ellis *et al* 1988). Perman *et al* (1978) reported no change in H₂ concentrations in 50 ml plastic syringes when analysis was performed within 8 hours, and also reported no change in H₂ concentration over 35 days when stored in 50 ml vacutainers. The procedure of withdrawing gas from the vacutainer for analysis, however, may result in significant loss of H₂ (Niu *et al* 1979).

Hydrogen analysis

Hydrogen concentrations in expired breath are commonly determined by gas chromatography. The traditional gas chromatographs use thermal conductivity detectors, and their sensitivity is relatively poor (minimal detectable concentrations range from 1-8 ppm) (Ostrander *et al* 1983). A more recent gas chromatograph analytical technique using a reduction gas detector has a superior sensitivity and is capable of determining H₂ concentrations to ± 0.010 ppm (Stevenson *et al* 1982, Ostrander *et al* 1983). Cheap and rapid H₂ analysis can also be made by electrochemical cells. Gas measurements using electrochemical cells have been shown to correlate well with the gas chromatography/thermal conductivity device ($r = 0.999$), and their accuracy is reported to be $\pm 2-3$ ppm (Bartlett *et al* 1980). More recently, smaller portable electrochemical cells (a pocket-sized breath H₂ analyser and a miniaturised desktop breath H₂ analyser), have been found to display adequate precision and sensitivity compared to the original stationary electrochemical cell (eg., GMI-exhaled monitor). In addition, they are cheaper and offer greater versatility (Braden *et al* 1993, Duan *et al* 1994).

Expiratory H₂ values are sometimes standardised ("normalised") to alveolar H₂ concentrations using carbon dioxide (CO₂) concentrations in breath as an internal standard. This is achieved by assuming a constant alveolar CO₂ concentration of 5%, and is based on the theory that the dilution of CO₂ and H₂ during collection procedures will be approximately the same given atmospheric CO₂ and H₂ concentrations are negligible (Niu *et al* 1979). The proposed advantages of this practice relate mainly to its ability to eliminate the effects of dead space dilution. These include: more reproducible H₂ breath measurements (eg., elimination of error induced by sampling different phases of expiration in end-expiratory sampling), the ability to compare results from different sampling methods, elimination of the need for difficult end-expiratory collections, and the identification of gas losses during storage by detecting abnormal

CO₂ values. Strocchi *et al* (1991) investigated the reproducibility of measuring gas concentrations in expired air and found that standardisation of the gases to constant CO₂ levels did not significantly reduce the variability of the measurements. He concluded that variability of gas concentrations between duplicate breath samples is mostly due to variable ventilation. Furthermore, some investigators feel that standardising expiratory H₂ concentrations may produce significant errors. Perman *et al* (1985) demonstrated that standardisation of breath samples is only appropriate under constant minute ventilation, as wide swings in ventilation rates result in a nonlinear relationship between CO₂ and H₂ (hyperventilation or hypoventilation will affect alveolar concentrations of H₂ more than CO₂). Therefore, standardising breath H₂ measurements in animals may cause significant errors due to the poor control of ventilation rates.

The standardisation of expiratory H₂ concentrations has also been investigated using N₂ and O₂ as internal standards (Robb and Davidson 1981, Davidson and Robb 1985). The standardisation of H₂ concentrations to constant O₂ concentrations (after the correction of H₂ to constant sample volume using N₂) was reported to reduce the average H₂ concentration range from 132% to 25% of the mean in consecutive samples (Robb and Davidson 1981). Davidson and Robb (1985) claimed that without O₂ standardisation of end-expiratory H₂ concentrations, a number of patients would have been interpreted to have sustained breath H₂ rises caused by variation in sample quality. In addition, endogenously produced carbon monoxide (CO) has been utilised as an internal standard for breath samples obtained from preterm and term infants (Stevenson *et al* 1982).

The standardisation of expiratory H₂ to constant CO₂ concentrations has been used in dogs to estimate the dilution of H₂ in collected breath samples due to dead space (Ludlow *et al* 1995). Despite the finding of a greater than 50% dilution of H₂ in mask collected samples, it has been shown that sample dilution does not necessarily interfere with the ability of the test to accurately predict carbohydrate malabsorption (Holland *et al* 1986 & 1989). However, to improve the sensitivity of the test, it is important to minimise dilution and to attempt to keep dilution rates constant during breath collection (ie., constant collection procedure, minimising excitement and exercise that may result in ventilation changes). The main indication for standardisation of expiratory H₂ appears to be to correct for sampling error due to sampling different phases of expiration. This is particularly important in paediatric patients where breathing patterns are frequently erratic and different phases of expiration are more likely to be sampled (Perman 1991).

Repeatability & individual variation of measured breath H₂ concentrations

It is well recognised that breath H₂ measurements following the administration of a test substrate vary markedly between individuals, even when the same dose of a nonabsorbable test solution is administered (Calloway and Murphy 1968, Bond *et al* 1972, Rumessen *et al* 1990). Bjorneklett and Jenssen (1980) have demonstrated that H₂ gas infused into the jejunum and colon via an endoscope results in different breath H₂ measurements between individuals, despite the same amount of H₂ gas being introduced. Therefore, individual variation of breath H₂ values may be related to differences in net intestinal H₂ production as well as to differences in the amount of H₂ diffusing into the portal circulation from the intestinal lumen. Greater individual variation in breath H₂ values were found after the introduction of larger amounts of H₂ into the colon, and has been suggested to occur because of differences in peristaltic activity between individuals in response to large gaseous loads (Bjorneklett and Jenssen 1980). Individual variability of breath H₂ excretion has also been shown to be present in dogs (Ludlow *et al* 1994a)

The repeatability of breath H₂ testing “within an individual” (reproducibility) has been assessed by evaluating the difference between duplicate samples, as well as evaluating the breath H₂ response on separate test days. The reproducibility of duplicate samples appears to be extremely good using the end-expiratory technique in man, with the average coefficient of variation between samples reported to be 5% in one study (Rumessen *et al* 1987). The repeatability of breath H₂ excretion on separate test days after the administration of the same type and amount of test substrate, also appears to be reasonable. Eight healthy human subjects given 50 g of lactulose were all found to have similar breath H₂ excretion on a second test day performed two weeks later (Levitt and Donaldson 1970), and peak H₂ and area under the breath H₂ vs time curve (AUC) results following a repeated 10 g lactulose load were found to deviate by a median of 20% (Rumessen *et al* 1990).

Reproducibility of the breath H₂ response to repeated doses of unabsorbable carbohydrates is likely to be reduced over time due to the induction of metabolic changes in the intestinal flora (Hertzler and Savaiano 1996, Florent *et al* 1985). Reduced reproducibility has also been reported when long time intervals occurred between different test periods (Rumessen *et al* 1990). Intra-dog variability of breath H₂ excretion has been assessed in 9 dogs over 3 days following the ingestion of a standard diet for 7 days prior to testing, and was not found to be significant (Ludlow *et al* 1994a). This indicates that measurement of H₂ excretion on one day is representative of H₂ excretion on all days given the diet provided before and during the test is consistent.

Applications of the Breath Hydrogen Test

Since the breath H₂ test was first developed in the late 1960's, it has been used for the investigation and diagnosis of carbohydrate malabsorption in various gastrointestinal disturbances as well as in the study of carbohydrate absorption in healthy individuals. Other applications have included gastrointestinal motility studies and the investigation of small intestinal bacterial overgrowth. The following section describes the clinical applications and research uses of the breath H₂ test in both human and veterinary medicine. Where appropriate, the advantages and disadvantages of this test in comparison to alternative tests used for the same purposes are also discussed.

Sugar Intolerance & Intestinal Disaccharidase Deficiencies

Lactose intolerance

Lactose intolerance develops when a deficiency in the brush border disaccharidase lactase, prevents the hydrolysis of lactose to its monosaccharide components, which is necessary before absorption can occur. Lactase deficiency in man may be primary (congenital or acquired) or secondary (Saavedra and Perman 1989, Ushijima *et al* 1995). A decrease in lactase activity at weaning (to approximately 10% of pre weaning levels), is considered a normal phenomenon in mammals and is thought to be due to a genetically determined decrease in lactase synthesis. Consequently, the acquired form of lactose intolerance is extremely common. Primary lactase deficiency has been reported in dogs (Hill 1972), and recently, the ability of dogs to assimilate lactose has been assessed by the breath H₂ technique (Sphor *et al* 1996).

The diagnosis of hypolactasia was the first clinical application of the breath H₂ test in man, and was introduced in the late 1960's and early 1970's shortly after its development (Calloway *et al* 1969, Levitt and Donaldson 1970). Early studies demonstrated the test to be at least as (and usually more) sensitive and specific than the lactose tolerance test (Levitt and Donaldson 1970, Metz *et al* 1975, Newcomer *et al* 1975). An increased breath H₂ concentration of >20 ppm over fasting values within two hours of ingesting 50g lactose, was shown to perfectly distinguish malabsorbers from absorbers (as defined by the lactose tolerance test), and this became the commonly used criterion of identifying significant sugar malabsorption by the breath H₂ technique (Metz *et al* 1975). A second study using very similar criteria (H₂ excretion >0.11 ml/min-which is equivalent to >22 ppm, within 2 hours after 50g of lactose), also demonstrated perfect separation of individuals with lactase deficiency from non-lactase deficient

individuals (Newcomer *et al* 1975). In this study breath H₂ results were compared to the measurements of lactase activity determined from small intestinal biopsies. Subsequent studies have varied the criteria for diagnosing significant sugar malabsorption, commonly changing the dose (eg., 1.75-2.0g/kg, maximum dose of 10, 20, 50g) and concentration (eg., 10-25%) of the sugar administered, the cut-off value for the rise in breath H₂ concentration (eg., 10, 15, 20 ppm, or cumulative concentrations), or the time period over which the rise is assessed (eg., 60, 90, 120, 150 minutes after ingestion) (Solomons and Viteri 1978, Bartlett *et al* 1980, Barr *et al* 1981, Davidson and Robb 1985, Strocchi *et al* 1993). This variation makes the results of the different studies difficult to compare. Generally, it is important to assess both the degree of the breath H₂ rise and the timing of this rise in relation to the dose and concentration of the sugar administered. Larger doses of sugars will result in more rapid breath H₂ rises due to faster small intestinal transit times created by the osmotic effect of the sugar. When the lactose breath H₂ test was compared to low lactase activity determined from small intestinal biopsies, sensitivities of 79-100% and specificities of 100% were reported (Metz *et al* 1975, Newcomer *et al* 1975, Barr *et al* 1981). Davidson and Robb (1985) even suggested that the lactose breath H₂ test may be a more sensitive test than determining lactase activity for the diagnosis of lactase deficiency, as the breath H₂ test more accurately predicted the response to low lactose diets when breath H₂ and lactase activity results were discrepant.

Other diagnostic tests commonly employed in children for detecting sugar malabsorption include the determination of faecal pH, detection of reducing sugars in the faeces, oral tolerance tests, ¹⁴C-labelled breath tests, and assays of disaccharidase activity in intestinal mucosal biopsies. Many of these tests are relatively insensitive: normal faecal pH does not rule out mild to moderate carbohydrate absorption, sugars are often rapidly metabolised in the colon and may therefore not appear in the faeces, and oral tolerance tests reflect the amount of sugar absorbed (rather than unabsorbed) and therefore commonly give false negative results when only small amounts of sugars are not assimilated (Levitt and Donaldson 1970). Even intestinal biopsy and assay of disaccharidase activity does not necessarily reflect the enzyme activity of the entire small bowel, and therefore may not always correlate well with the patients clinical signs (Perman *et al* 1978, Davidson and Robb 1985).

The use of breath H₂ testing for the diagnosis of lactase deficiency (and other disaccharidase deficiencies) offers several advantages over the tests mentioned above. It is a non-invasive, well tolerated, easy and inexpensive procedure to perform. Sample storage and shipment of samples is possible, analysis is rapid, results are not adversely affected by intermediary glucose metabolism, there is no need for blood sampling or chemical isotopes, the test is indicative of the functional capacity of the entire small intestine to assimilate the sugar,

and very good sensitivities and specificities have been documented (Metz *et al* 1975, Newcomer *et al* 1975, Barr *et al* 1981). Although intestinal biopsy with assay of disaccharidase activity remains the gold standard for diagnosing lactase deficiency, the breath H₂ test has become the most accurate indirect and non-invasive test for investigating sugar intolerance. While false positive results are uncommon, false negative results occasionally occur, and usually reflect a poor ability to excrete H₂ (see "Variables and limitations of the breath hydrogen test") (Barr *et al* 1981). Malabsorption of lactose detected by breath H₂ does not necessarily indicate *primary* lactase deficiency, as abnormal breath H₂ responses may also be seen with glucose and galactose malabsorption, and lactase deficiency secondary to gastrointestinal mucosal injury. Additional breath H₂ tests using glucose and galactose solutions can be used to assess the absorption of these monosaccharides (Solomons and Viteri 1978), and a sucrose solution can also be administered to determine if more than one type of disaccharidase is deficient. Concurrent deficiency of two disaccharidases (including lactase) indicates secondary rather than primary lactase deficiency.

Sucrose intolerance

Similar breath H₂ tests to the protocols used for assessing lactose intolerance have also been applied to the diagnosis of sucrase-isomaltase deficiency in humans (Metz *et al* 1976b, Perman *et al* 1978, Davidson and Robb 1985). Although this condition is uncommon, several forms of sucrase-isomaltase deficiency exist, and the activity of sucrase is usually the most severely reduced. Sucrose is normally efficiently digested and absorbed in healthy individuals, and the malabsorption of sucrose following a 50g oral sucrose load may be detected by breath H₂ testing. Several studies which have assessed biopsy-proven sucrase-isomaltase deficient individuals by the breath H₂ technique have displayed greater than 20 ppm rises over fasting values within 3 hours of testing (Metz *et al* 1976b, Perman *et al* 1978, Bartlet *et al* 1980).

As for lactase deficiency, an abnormal breath H₂ response to sucrose may be due to a primary or secondary sucrase deficiency. A secondary sucrase deficiency has been demonstrated in two children using the breath H₂ test, both of whom responded to a sucrose free diet and later became tolerant to sucrose (Davidson and Robb 1985).

Fructose & Sorbitol intolerance

Fructose and sorbitol are both commonly used industrial sweeteners. Breath H₂ measurements taken after the ingestion of these sugars have demonstrated that their assimilation in the small intestine is limited, with malabsorption of these sugars occurring in a large percentage of individuals (Ravich *et al* 1983, Fernandez *et al* 1993).

Small Intestinal Bacterial Overgrowth

A variety of medical conditions can predispose to proliferation of small intestinal bacteria. This occurs either by mechanisms resulting in a failure to clear bacteria from the upper gastrointestinal tract or, less commonly, by continuous seeding of the upper gastrointestinal tract with colonic bacteria (King and Toskes 1979). The use of breath H_2 in the diagnosis of small intestinal bacterial overgrowth (SIBO) is based on the premise that the abnormally large numbers of bacteria present in the small intestine ferment ingested carbohydrates resulting in an early increase in breath H_2 excretion. Although bacterial numbers in the small intestine with SIBO are usually much less than bacterial numbers in the colon, H_2 produced by bacteria in the small intestine is more likely to be excreted in the breath than is colonic derived H_2 . Hydrogen produced in the small intestine is less likely to be expelled as flatus, and has shown to be more efficiently absorbed than H_2 produced in the colon (Metz *et al* 1976c, Bjorneklett and Jenssen 1980). In theory, because H_2 excretion in the breath occurs rapidly after the onset of substrate fermentation, H_2 produced in the small intestine should be readily distinguishable from H_2 produced in the colon (Rhodes *et al* 1979).

A large increase in small intestinal H_2 production due to SIBO was first identified in a post gastrectomy patient after lactose was infused into the small intestine (Levitt 1969). Since this time, several studies have attempted to evaluate the use of breath H_2 testing in the diagnosis of SIBO in man. In the majority of studies, glucose and lactulose are used as test substrates and breath samples are collected every 10-30 mins for 2-4 hours after the test substrate is administered by mouth. The main disadvantage of using completely absorbable sugars (eg., glucose) is that false negative test results may occur when bacterial colonisation is distal to the site of substrate absorption (Metz *et al* 1976c). Lactulose or other non-absorbable sugars can be used to evaluate bacterial overgrowth over the entire length of the small intestine. However, interpretation of the breath H_2 response using non-absorbable sugars can be difficult. The "double peak" phenomenon of an early breath H_2 rise (small intestinal H_2 production) distinguishable from a later sustained breath H_2 rise (colonic H_2 production) after the ingestion of lactulose is considered to be indicative of SIBO (Rhodes *et al* 1979). Unfortunately, an early rise may not always be distinguished, particularly if the small intestinal transit of the substrate is rapid, a delay in the appearance of H_2 in the breath after fermentation occurs, or bacterial overgrowth is present in the distal ileum (Rhodes *et al* 1979, Kerlin and Wong 1988, Riordan *et al* 1996). The sensitivity of the glucose or lactulose breath H_2 test for diagnosing SIBO may also be reduced when an individual's ability to produce H_2 is poor, or if the bacteria of the small intestine do not produce H_2 (King and Toskes 1986). In addition to the glucose and lactulose breath H_2 test, a rice flour breath H_2 test (50g rice flour in the form of pancakes) has been

evaluated for diagnosing SIBO (Kerlin and Wong 1988). Unfortunately, results using the rice substrate were inferior to those obtained with the glucose breath H₂ test.

The use of breath H₂ testing to investigate SIBO may occasionally give false positive results. An early breath H₂ rise due to oral fermentation and/or stimulation of the gastro-ileal reflex (increased ileal output of digesta which occurs with eating) could be interpreted as SIBO by either the glucose or lactulose breath H₂ test. In addition, when absorbable sugars are used as the test carbohydrate, false positive tests may result from the malabsorption of these sugars due to rapid small intestinal transit times or small intestinal disease. For example, significant malabsorption of 100 g test doses of glucose have been demonstrated in partially gastrectomised patients with diarrhoea (Bond *et al* 1972). With non-absorbable sugars, false positive tests may result from rapid gastrointestinal transit times, or an atypical delivery pattern of the sugar to the colon which results in a double peak of breath H₂ excretion without small intestinal fermentation occurring (Riordan *et al* 1996).

In defining a positive breath H₂ test for SIBO, various criteria have been applied to the breath H₂ results from different studies (see Table 2.1). With the glucose breath H₂ test, any rise in breath H₂ should be considered abnormal if physiological doses are used and glucose does not reach the colon. Evidence for a stable rise should also be present (i.e., rise sustained for at least 2 subsequent readings) to reduce the chance of fluctuations in breath H₂ levels resulting in a false positive diagnosis. In contrast, the definition of a positive lactulose breath H₂ test for SIBO has involved demonstrating separate small intestinal and colonic peaks of breath H₂ excretion. Recently, the use of this “double peak” definition after the administration of lactulose has been evaluated in conjunction with scintigraphy, and its occurrence was found to be both an insensitive and non-specific test for SIBO (Riordan *et al* 1996). Table 2.1 contains a summary of the criteria used and the sensitivity and specificity (obtained in comparison to quantitative culture results of duodenal aspirates) of various breath H₂ studies investigating SIBO in man, as well as one study performed in dogs. The duration of the test period over which breath samples have been collected has also been shown to be important. Kerlin and Wong (1988) found that extending a glucose or rice flour breath test from 2 to 4 hours, reduced the specificity of the test without any increase in sensitivity.

High basal (fasting) breath H₂ measurements have been found to be associated with the presence of SIBO in man, and therefore fasting breath H₂ values are often assessed in the investigation of this condition (Perman *et al* 1984, Kerlin and Wong 1988, Corazza *et al* 1990, de Boisseu *et al* 1996). When fasting breath samples are interpreted in conjunction with the presence of an early breath H₂ rise, they have been found to be 100% specific for SIBO, but the

Table 2.1 Examples of the methodology and test criteria used for investigating SIBO by breath H₂ analysis

<i>Study</i>	<i>Subjects</i>	<i>Test substrate</i>	<i>Criteria for a positive breath H₂ test</i>	<i>Criteria for a positive quantitative culture of jejunal aspirate</i>	<i>Sensitivity & specificity of the breath H₂ test</i>
Metz <i>et al</i> 1976	17 suspect SIBO patients	50g glucose	peak breath H ₂ of 20 ppm or more	> 10 ³ bacteria/ml	83% sensitivity 100% specificity
Rhodes <i>et al</i> 1979	37 controls 27 suspect SIBO patients	10g lactulose	breath H ₂ >20 ppm, at least 15 minutes before colonic peak	> 10 ⁴ bacteria/ml	90% sensitivity 100% specificity
King & Toskes, 1986	10 diarrhoea patients 20 culture +ve for SIBO	A. 10g lactulose B. 80g glucose	A. 2 consecutive samples >10 ppm over baseline, separate from second rise >20 ppm. B. 2 consecutive samples >20 ppm over baseline	> 10 ⁶ bacteria/ml	A. 61% sensitivity 100% specificity B. 83% sensitivity 100% specificity (three H ₂ non-producers not included)
Kerlin & Wong, 1988	45 suspect SIBO patients 20 controls	A. 50g glucose B. 50g rice flour	cut-off value based on mean ± 2SD of controls: A. 12 ppm, B. 14 ppm.	> 10 ⁵ bacteria/ml	A. 93% sensitivity 78% specificity B. 81% sensitivity 67% specificity
Corazza <i>et al</i> 1990	44 diarrhoea patients: 35 lactulose, 20 glucose	A. 12g lactulose B. 75g glucose	A. 2 consecutive samples >10 ppm over baseline, separate from second rise >20 ppm B. >10 ppm rise over baseline	> 10 ⁶ bacteria/ml	A. 68% sensitivity 44% specificity B. 62% sensitivity 83% specificity
Riordan <i>et al</i> 1996	28 suspect SIBO patients	10g lactulose & 20 mbq T _c	A. 2 consecutive samples > 10 ppm over baseline, separate from second rise >20 ppm, OR >10 ppm rise over baseline within 20 mins & 15 mins before second rise B. 2 consecutive samples >10 ppm, > 10 mins prior to increased caecal radioactivity	>10 ⁵ bacteria/ml OR >10 ³ bacteria/ml enterobacteraciae OR >10 ² bacteria/ml bacteroides or clostridium	A. 17% sensitivity 70% specificity B. 40% sensitivity 100% specificity
de Boissieu <i>et al</i> 1996	15 controls 6 culture +ve for SIBO	1g/kg glucose	>10 ppm over baseline	N/A	100% sensitivity 100% specificity
Rutgers <i>et al</i> 1996	107 chronic diarrhoea dogs	mixed sugars including lactulose	>5 ppm fasting breath H ₂ and/or 2 consecutive readings >6 ppm in 2 hours	>10 ⁵ total or >10 ⁴ anaerobic bacteria/ml	63% sensitivity 73% specificity

SIBO = small intestinal bacterial overgrowth; ppm = parts per million; SD = standard deviation; T_c = technetium; N/A = not applicable; +ve = positive

sensitivity reported was poor (Kerlin and Wong 1988). A high fasting breath H₂ reading on its own may not be specific for SIBO, as other causes of carbohydrate malabsorption and the composition of meals ingested prior to testing have been shown to influence the fasting breath H₂ values in man and dogs (Corazza *et al* 1987, Washabau *et al* 1986b, Kotler *et al* 1982).

Quantitative cultures of small intestinal aspirates are considered to be the "gold standard" for the diagnosis of SIBO (King and Toskes 1979). Consequently, the evaluation of breath H₂ tests are commonly compared with quantitative culture results of duodenal aspirates. In comparison to quantitative culture results, the glucose and lactulose breath H₂ tests have reported sensitivities of 62-100% and 17-90%, and specificities of 78-100% and 44-100% respectively (Metz *et al* 1976c, Rhodes *et al* 1979, King and Toskes 1986, Kerlin and Wong 1988, Corazza *et al* 1990, Riordan *et al* 1996, de Boissieu *et al* 1996). The sensitivity of the glucose breath H₂ test appeared to be greater when higher doses of glucose were administered per os (King and Toskes 1986), but this practice may reduce the specificity of the test. The poor correlation of the breath H₂ test and quantitative culture of intestinal aspirates in some studies may not always be due to inaccurate breath H₂ results. Considerable error may exist with the diagnosis of SIBO based on the quantitative culture technique, as small intestinal aspirates are only representative of the area from which the sample of chyme for culture is collected, and the duration of fasting prior to sampling, the contents of the previous meal, and differences in collection and culture techniques may all affect culture results.

In recent years, SIBO has been documented in dogs (Rutgers *et al* 1995, Batt *et al* 1991, Williams *et al* 1987, Simpson *et al* 1990). The majority of reports of SIBO in dogs have included German Shepherd dogs, dogs with exocrine pancreatic insufficiency, and dogs with chronic gastrointestinal disorders. The breath H₂ test for diagnosing SIBO has been evaluated in this species on one occasion (Rutgers *et al* 1996). In this study, dogs with suspected SIBO were given a mixed sugar load (including lactulose) orally, and the results of the breath H₂ test were compared to quantitative cultures of duodenal aspirates. The sensitivity and specificity of the breath H₂ test was reported to be 63% and 73% respectively. However, these results need to be interpreted with caution, as the lactulose breath H₂ test has been shown to be difficult to interpret in people (Riordan *et al* 1996), and acceptable cut-off values for quantitative culture results of duodenal aspirates for healthy dogs have recently been challenged (Simpson 1994, Davenport 1996).

Alternative methods for investigating SIBO include assays of serum folate and cobalamin, assays of serum unconjugated bile acids, urinary excretion of bacterial metabolites (eg., indican, phenols, PABA), and labelled CO₂ breath tests (¹⁴C-bile acid, ¹⁴C-xylose) (King

and Toskes 1979 & 1986, Williams and Guilford 1996). Of these tests, the ^{14}C -xylose breath test and the assay of serum unconjugated bile acids are probably the most accurate indirect methods of screening for SIBO in humans. However, most of these tests are laborious to perform and expensive, as well as not being widely available or feasible to perform in veterinary practice. The other tests used generally display disappointing sensitivity and specificity. Urinary excretion of bacterial metabolites and abnormal ^{14}C -bile acid breath tests may be seen with other forms of malabsorption. Serum folate and cobalamin assays are the tests available to most general practitioners, but their sensitivity is poor and measurements are strongly influenced by the diet (Davenport *et al* 1994).

The need for a simple and non-invasive test with the ability to assess the entire length of the small intestine in SIBO makes breath H_2 testing an exciting prospect for the investigation of SIBO. Unfortunately, both the glucose and lactulose breath H_2 tests have occasionally displayed disappointing sensitivities and specificities (Corozza *et al* 1990). In particular, after lactulose is administered as an aqueous solution breath H_2 may display extremely poor sensitivity, even when the test is combined with scintigraphy and poor H_2 producers have been excluded (Riordan *et al* 1996). Therefore, the need for further investigation into the diagnosis of SIBO by breath H_2 using alternative test substrates is required. The ideal test substrate for investigating SIBO is probably a rapidly fermentable, non-absorbable sugar that does not have a rapid gastrointestinal transit time. A non-absorbable, but rapidly fermentable substrate would allow the investigation of bacterial overgrowth along the entire length of the small intestine, and accurate assessment of the timing of the rise in breath H_2 rise (minimal delay between fermentation and appearance of breath H_2 in the breath). Slower small intestinal transit times would help to separate H_2 production from the small and large intestine.

Gastrointestinal Motility Studies

Breath H_2 testing has been utilised to assess upper gastrointestinal motility in humans, cats and dogs (Bond *et al* 1975, Muir *et al* 1991, Papasouliotis *et al* 1995). The time measured from the ingestion of a fermentable substrate to the beginning of the breath H_2 rise can be used to estimate the patients' orocolic/orocaecal transit time (OCTT). Assessment of OCTTs are useful for a number of reasons. They can be used to study the physiology of small bowel motility in disease, such as in malabsorptive disorders (Papasouliotis *et al* 1993a, Metz *et al* 1976d) and functional bowel disorders (Cann *et al* 1983, Armbrrecht *et al* 1986), and can also be used to assess the effect of drugs (Staniforth and Rose 1989, Sparkes *et al* 1996), diet (Read *et al* 1980, Papasouliotis *et al* 1993b), or other external stimuli (Thompson 1989) on upper gastrointestinal motility.

The beginning of a sustained breath H_2 rise after the consumption of a meal or test solution, is thought to accurately assess the time the head of the meal/solution enters the colon. The lag time between the caecal appearance of a rapidly fermentable substrate (eg., lactulose) and the appearance of H_2 in the breath is approximately 4-5 minutes (Bond *et al* 1975). A number of studies have shown that the beginning of a sustained breath H_2 rise correlates well with (i) the arrival of a non-absorbable marker at the distal ileum (detected by gastrointestinal intubation) (Bond *et al* 1975), (ii) increased radioactivity over the caecum (determined by scintigraphy) (Read *et al* 1980, "85, Caride *et al* 1984, Madsen *et al* 1991), (iii) the arrival of radiopaque markers in the caecum (detected by fluoroscopy) (Armbrecht *et al* 1986), and (iv) the arrival of barium at the caecum (detected by fluoroscopy) (Hirakawa *et al* 1988). Some studies have demonstrated that the time to reach the maximum (peak) breath H_2 values also correlate well with OCTTs (Bond *et al* 1975, Read *et al* 1980). Read *et al* (1980) found a good correlation between the time of occurrence of peak breath H_2 levels and the time when maximum radioactivity was recorded over the colon. The authors suggested that the timing of the peak could therefore be used to estimate when the bulk of the test meal/substrate entered the colon. However, this finding was not repeatable (Read *et al* 1985), and the relationship of peak breath H_2 and substrate transit probably varies with the pattern of entry of substrates into the colon, and the substrate's fermentability. Although the rise of breath H_2 most likely begins when the substrate enters the colon, the time of the breath H_2 rise is often referred to as the oro-caecal transit time rather than the oro-colic transit time. This nomenclature exists because many alternative methods of assessing upper gastrointestinal motility measure the time the substrate arrives at the level of the caecum, and have been shown to correlate well with the breath H_2 method.

The estimation of OCTT by breath H_2 is dependent on a number of different factors. These include the subjects tested (eg., healthy vs diseased), type and concentration of test substrates administered (transit time and H_2 production may vary), the frequency of breath sampling, and the criteria used to determine the time when the test substrate arrives at the colon. Frequently, non-absorbable carbohydrates (eg., lactulose) are employed as test substrates to ensure that an identifiable increase in H_2 excretion occurs. Orocolic transit time has been estimated with lactulose administered as an aqueous solution (Bond *et al* 1975, Caride *et al* 1984), in combination with a liquid or semi-liquid meal (La Brooy *et al* 1983, Sarno *et al* 1993), and mixed with solid food (Read *et al* 1980, Staniforth and Rose 1989, Madsen *et al* 1991). Carbohydrate-containing meals without the addition of non-absorbable substrates such as lactulose, have also been used to assess OCTT (Read *et al* 1985, Armbrecht *et al* 1986, Papasoulitotis *et al* 1993b). The use of lactulose administered as an aqueous solution to

determine OCTT has several disadvantages. Lactulose is a non-physiological substrate, which due to its non-absorbable and osmotic nature causes a dose dependant acceleration of small intestinal transit (Levitt and Donaldson 1970, Bond *et al* 1975). For these and other reasons (eg., liquid nature, lack of other nutrients that delay transit) it is not surprising that lactulose administered as an aqueous solution does not provide a useful index of the transit of food (Read *et al* 1980, Madsen *et al* 1991, Sarno *et al* 1993). Furthermore, large variations in OCTT, both between and within individuals, occurs when lactulose is administered as an aqueous solution (La Brooy *et al* 1983). It has been shown that this variability is largely due to the influence of different phases of the motor migrating complex occurring in the upper gastrointestinal tract when lactulose is ingested (Di Lorenzo *et al* 1991). However, when lactulose is administered with a liquid or solid meal the reproducibility of the test is greatly improved; most likely due to the induction of gastrointestinal motility patterns associated with feeding (La Brooy *et al* 1983, Staniforth and Rose 1989, Di Lorenzo *et al* 1991). In animals, OCTT has been measured after the administration of aqueous lactulose in cats (Muir *et al* 1991), lactulose with a semi-liquid meal in cats (Papasouliotis *et al* 1993a, Sparkes *et al* 1996), and a mixed solid meal (without lactulose) in dogs (Papasouliotis *et al* 1993b, *et al* 1995).

For accurate determination of OCTTs using breath H_2 , the collection of breath sampling needs to be frequent. Most studies use a sampling intervals of 5-15 minutes. Breath samples are usually collected over 3-5 hours or until the breath H_2 values have risen significantly. Therefore, the time period over which breath samples are collected is largely dependant on the type of test substrate administered.

Various criteria have been used to define OCTT by breath H_2 testing, and some examples of these are shown in Table 2.2. A comparison of lactulose breath H_2 tests by Sarno *et al* (1993) identified that the criteria used to define OCTT, rather than the methodology (dose and concentration of lactulose), accounted for the greatest variation of OCTTs reported between different studies. In many of the early breath H_2 studies, the definitions of OCTT were vague (eg., "first detectable rise in breath H_2 ", "a sustained increase in H_2 concentrations"), and probably relied on visual assessment of the breath H_2 curve. If interpreters are experienced, this method is one of the most accurate ways of assessing OCTT by breath H_2 analysis (Sarno *et al* 1993). More complicated criteria have been applied to reduce the subjectivity of the visual test. Many workers use baseline references (ie., a percentage rise from baseline, a certain ppm rise over baseline) to define the colonic entry point of the test meal/solution (Read *et al* 1985, Hirakawa *et al* 1988, Staniforth and Rose 1989, Schering *et al* 1993). Frequently, the definition also includes criteria which demonstrate that the rise over baseline is sustained for a certain period of time (or number of readings) before the rise can be attributed to the substrate entering

Table 2.2 Examples of methodology & criteria used for estimating OCTT by breath H₂ analysis

<i>Study</i>	<i>Subjects</i>	<i>Test meal/solution</i>	<i>Criteria used to define OCTT</i>	<i>Mean/median OCTT (minutes)</i>
Bond & Levitt, 1975	Healthy people	10% lactulose solution (5,10,20g)	"Earliest breath H ₂ rise"	5g dose= 128 10g dose= 94 20g dose= 40
Caride <i>et al</i> 1984	Healthy people	Iso-osmotic lactulose solution (10g)	Sustained increase in 3 consecutive samples, at least a 10 ppm increase between any 2 of these	75.1
Read <i>et al</i> 1985	Healthy people	Solid test meal (containing baked beans)	A. Double baseline B. 3 ppm rise over baseline C. 10 ppm rise over baseline	A. 247 B. 240 C. 303
Armbrecht <i>et al</i> 1986	Healthy people (others not included)	Solid test meal (porridge)	A. First of 3 consecutive increased values B. H ₂ values > 50% of 90 & 120 minute values	A. 192 B. 238
Staniforth & Rose, 1989	Healthy people	13.4g lactulose: A. as solution B. with rice pudding	15 ppm rise over baseline	A. 86.4 B. 98.6
Madsen <i>et al</i> 1991	Healthy people	Mixed solid & liquid meal (bread, cheese, yoghurt, water) & 20g lactulose	Sustained rise of 10 ppm or more over baseline	86.3
Sarno <i>et al</i> 1993	Healthy people	A. 15g lactulose, 10% solution B. 15g lactulose in semi-liquid meal	Rule set involves: a. calculating moving medians b. identifying local sustained rise c. identifying point of significance d. identifying global sustained rise (and others compared)	A. 77.9 B. 143.3
Muir <i>et al</i> 1991	Healthy cats	A. Xylose .75g/kg B. 3.35g lactulose	When cusum value for H ₂ >0.5 mls/ hour (>4.5 ppm) over baseline, sustained for at least 3 readings	A. no significant H ₂ values B. 86
Schlesinger <i>et al</i> 1993	Hyperthyroid cats: A. pre treatment B. post treatment	5.3g lactulose	When rise >4 ppm over baseline, sustained for at least 3 readings	A. 27.7 B. 56.5
Papasouliotis <i>et al</i> 1993a	A. Healthy cats B. Hyperthyroid cats	Liquid meal (3ml/kg) & 1g/kg lactulose	When cusum value for H ₂ >0.5 mls/ hour over baseline, sustained for at least 3 readings	A. 98 B. 53
Papasouliotis <i>et al</i> 1995	Healthy dogs	Solid meal (canned dog food)	When cusum value for H ₂ >5 ppm over baseline, sustained for 3 readings	135

the colon). This helps to reduce the chance of breath H₂ fluctuations or an early rise in breath H₂ (oral fermentation and/or gastroileal reflex) being mistakenly defined as the colonic entry point. Brown *et al* (1987) used a liquid bean meal and a liquid lactulose labelled meal in rats to demonstrate that a rise of 2 ppm or more over baseline, sustained for at least three readings accurately determined the presence of the test substrate in the caecum in 91% of animals, but in 9% of animals the test substrate had arrived at the caecum without a rise in H₂ concentrations.

The problems with using set criteria for defining OCTT are several fold. Different values of H₂ production with different substrates or different doses of the same substrate, makes extrapolation of such criteria to other test substrates or doses inaccurate. Errors may occur because of markedly different breath H₂ measurements between individuals (eg., H₂ non-producers), when high baseline breath H₂ values are present, or when an early breath H₂ rise (oral flora/gastroileal reflex or SIBO) occurs. Samo *et al* (1993) have recently developed a set of rules to define the colonic entry point of lactulose administered as a semi-liquid meal, which has been shown to correlate well with visual assessment of the breath H₂ curve. However, this technique is relatively complicated (see Table 2.2). Most of the studies of OCTT in animals have applied cusum analysis (plotting a cumulative sum chart), which sums the progressive deviation of the breath H₂ readings from baseline, to emphasise and therefore more accurately detect the point at which breath H₂ concentrations increase (Muir *et al* 1991, Papasouliotis *et al* 1993a, *et al* 1995, Sparkes *et al* 1996). This technique compares reasonably well with OCTTs defined by the sulphasalazine method (Papasouliotis *et al* 1995), and a highly significant correlation has been reported between this technique and visual assessment or baseline reference techniques (Sparkes *et al* 1996).

Because breath H₂ testing is a simple, non-invasive, and inexpensive test, and does not involve radiation exposure, it is a useful alternative to the other commonly used methods of assessing upper gastrointestinal motility (eg., non absorbable markers with direct sampling by gastrointestinal intubation, barium sulphate, radiopaque markers, scintigraphy). The validity of the breath H₂ test in determining OCTTs has been compared with all of the above mentioned alternative tests in man, and OCTT estimates were found to correlate extremely well with the breath H₂ technique in all cases. The main disadvantage of the breath H₂ test in comparison to some of these other tests, is that gastric emptying is not able to be assessed independently of the small intestinal transit time. The accuracy of the breath H₂ test may be compromised by the presence of significant numbers of bacteria in the terminal ileum which may result in an underestimation of OCTT (Armbrecht *et al* 1986), and by the presence of SIBO or other causes of an early rise in breath H₂ values (Samos *et al* 1993).

Ideally, OCTT determination by the breath H₂ test should utilise a physiological substrate, preferably a solid meal of homogenous consistency which contains an indigestible but rapidly fermentable carbohydrate source (eg., lactulose). By using a meal of homogenous consistency, separation of the meal into solid and liquid phases is less likely. Therefore, similar gastric emptying rates of meal components will occur, and increases in breath H₂ values will more accurately assess the entry time of the head of the entire meal as it passes into the colon (Read *et al* 1980). The use of a solid meal is more likely to represent the normal daily OCTT of the subject tested, and is more reproducible than OCTTs measured after an aqueous lactulose solution. The addition of a non-absorbable, rapidly fermentable carbohydrate maximises the likelihood of significant breath H₂ excretion and reduces the lag time between colonic entry of the substrate and breath H₂ excretion. When set criteria are applied to breath H₂ data to determine the OCTT, they should always be assessed in conjunction with the visual method of assessing the breath H₂ curve. In this way, obvious errors or inadequacies of the set criteria are not overlooked. The most appropriate criteria to use when defining the colonic entry point of a test substrate is controversial, but should ideally involve criteria that have been shown to correlate well with other acceptable techniques for assessing OCTT (given that the same test substrate and dose is applied).

Investigation of carbohydrate malabsorption in diseased individuals

Carbohydrate malabsorption may develop as a consequence of a variety of gastrointestinal disorders due to an interruption in the normal degradation and absorptive processes of this nutrient (eg., loss of pancreatic amylase action, primary or secondary deficiencies of intestinal brush border enzymes and carrier proteins, presence of abnormal gastrointestinal motility). In dogs, the principle causes of carbohydrate malabsorption are exocrine pancreatic insufficiency (EPI) and small intestinal disease (Williams and Guilford 1996). When significant carbohydrate malabsorption is present, clinical signs such as abdominal discomfort, borborygmus, flatulence, diarrhoea and weight loss may develop. While small intestinal malabsorption due to EPI can now be readily and accurately diagnosed by the serum trypsinogen like immunoreactivity (TLI) assay, the diagnosis of other causes of small intestinal malabsorption requires small intestinal tissue from biopsies, or the use of a variety of mucosal malabsorption tests. Histological examination of small intestinal tissue is the most commonly used method of diagnosing small intestinal disease. However, non-invasive methods of obtaining small intestinal biopsies via gastroduodenoscopy are not always available in clinical practice, and morphological changes are not apparent in many animals with small intestinal disease (Batt and Hall 1989, Williams and Guilford 1996).

Several tests of malabsorption have been developed to investigate small intestinal function and identify the presence of significant small intestinal malabsorption. The more commonly used tests in veterinary medicine include: microscopic examination of faeces for the presence of undigested food; quantitative faecal fat determination; the xylose absorption test; serum cobalamin and folate assays; and more recently breath H₂ testing. Many of these tests display poor sensitivities which limit their application in clinical practice. Microscopic examination of the faeces for undigested food is subjective, imprecise, and the results are affected by the type of food consumed. Furthermore, starch granules are usually degraded by the colonic microflora and thus may not appear in the faeces despite the malabsorption of starch (Jacobs *et al* 1989). Although the quantitative faecal fat test is the most accurate test for diagnosing steatorrhoea, fat malabsorption is not always a feature of small intestinal disease, and therefore the sensitivity of this test can be poor (Williams and Guilford 1996). Other disadvantages of the quantitative faecal fat test are that it is cumbersome to perform, unpleasant for the clinician and expensive. The xylose absorption test also displays disappointing sensitivities in both the dog and cat (Hawkins *et al* 1986, Washabau *et al* 1986b). Results of this test can be affected by varying gastric emptying times and intestinal motility, altered small intestinal blood flow, the presence of intraluminal bacteria, ascites, and renal insufficiency, and the extent of the lesion present (Jacobs *et al* 1989, Williams and Guilford 1996). Serum cobalamin and folate concentrations are considered to be more sensitive and specific tests of malabsorption than the xylose absorption test, but inadequacies also exist with these assays (Williams and Guilford 1996). Reduced folate concentrations occur only with disease of the proximal small intestine, and only when the disease is severe or has been present long enough to deplete body folate stores. Similarly, intestinal disease needs to be severe and present for sufficient periods before cobalamin depletion occurs. Serum folate and cobalamin values are also greatly influenced by the diet (Davenport *et al* 1994).

Breath H₂ testing has been used to investigate carbohydrate malabsorption in people with various gastrointestinal diseases, and has been found to compare favourably with other tests of small intestinal malabsorption. Breath H₂ performed on gastrectomised patients with intermittent diarrhoea after the ingestion of 100g glucose, was found to be a more sensitive test of malabsorption than the glucose absorption test (Levitt and Donaldson 1970). Application of the lactose breath test to children with suspected lactase deficiency has been extensive, and shown to be more accurate than the lactose absorption test (Levitt and Donaldson 1970, Newcomer *et al* 1975, Barr *et al* 1981). Mackie *et al* (1981) used a test meal of rice flour (100 g) in the form of pancakes fed to 5 patients with exocrine pancreatic insufficiency, and showed that all patients clearly malabsorbed the meal with a mean breath H₂ peak of 101ppm. The rice

flour breath H₂ test has since been used with success in several studies of carbohydrate malabsorption (Kerlin *et al* 1984, Ladas *et al* 1989, Ladas *et al* 1993). Kerlin *et al* (1984) evaluated 23 healthy subjects, 17 subjects with chronic pancreatic disease and 22 subjects with small intestinal disease, and calculated the upper limit of normal H₂ production to be 20 ppm (based on the mean peak breath H₂ concentration + 2 standard deviations from this mean). Using this cut-off value for the diagnosis of clinically significant carbohydrate malabsorption, 70% and 73% of the patients with chronic pancreatic disease and small intestinal disease respectively, were found to malabsorb the rice flour. This sensitivity compared favourably with the 3 day quantitative faecal fat excretion test, and the specificity was found to be 94% based on healthy and diseased controls. In agreement with these findings Ladas *et al* (1989) identified twice as many patients with small intestinal resection or disease as having significant small intestinal malabsorption using the rice flour breath H₂ test in comparison to faecal fat assessment. Breath H₂ tests have also been reported to more accurately predict the clinical severity and response to dietary therapy than small intestinal biopsy specimens (including determination of brush border enzyme activity) in the investigation of children with post gastroenteritis syndrome, chronic non-specific diarrhoea, and failure to thrive syndromes (Davidson and Robb 1985). In this study, sucrose and lactose breath H₂ tests were reported to accurately predict the clinical severity and response to dietary change in 95.6% of cases, in comparison to the 77.1% of cases accurately predicted by small intestinal biopsy results.

Breath H₂ testing has also been used to investigate small intestinal malabsorption in animals. Breath H₂ testing after xylose administration, and the ingestion of a solid meal (Prescription diet d/d; Hill's Pet Products, Topeka, Kansas), showed significantly higher mean fasting and peak breath H₂ values in dogs with EPI and chronic small intestinal disease in comparison to healthy controls (Washabau *et al* 1986b). This study also showed that the sensitivity of this test was superior to the xylose absorption test. Muir *et al* (1994) investigated 5 cats with chronic diarrhoea and/or vomiting and weight loss, and 11 healthy cats following the administration of xylose, and also found the breath H₂ test to be superior to the xylose absorption test. All the diseased cats displayed greater breath H₂ excretion than the mean breath H₂ excretion of the healthy cats at all of the time points that samples were analysed. Another study of breath H₂ testing with xylose in healthy and diseased cats (chronic intestinal disease), showed excellent specificity of the breath H₂ test (no healthy cats had significantly increased breath H₂ values over the monitoring period). Large sustained breath H₂ increases in more than 50% of diseased cats occurred, including cats with no abnormalities on intestinal biopsies (Papasouliotis *et al* 1994). Significantly increased breath H₂ concentrations have also been measured in calves with chloramphenicol induced small intestinal damage (Holland *et al* 1986),

and in calves with cryptosporidium infections (Holland *et al* 1989). In horses, elevated breath H₂ levels were detected in 5 of 9 horses with chronic diarrhoea or weight loss, but an abnormal xylose absorption test was only detected in one of these cases (Bracher *et al* 1995).

As discussed above, it appears that the breath H₂ test is more sensitive and specific than the xylose absorption test in detecting small intestinal malabsorption in veterinary patients. However, false negative results have been identified using this test in man (Kerlin *et al* 1984, Ladas *et al* 1989) as well as in animals (Papasouliouios *et al* 1994). False negative breath H₂ tests in man are often related to the presence of poor H₂ producers. This may be an individual phenomenon related to the types and activities of colonic microbes present, or may be induced from conditions which alter colonic microbial populations eg., antibiotic therapy, acidic colonic environment, preparation for colonoscopy (see "Variables and limitations of breath H₂ measurements"). The incidence of poor H₂ producers is unknown in dogs. However, the incidence may be less than in humans, as this condition is largely related to high CH₄ producing ability, which is thought to be uncommon in dogs.

Although the breath H₂ test appears to be a very promising one for the diagnosis of small intestinal malabsorption in dogs and cats, only limited data is available at present. Xylose has been the most commonly used breath H₂ test substrate to date, with breath samples collected every 15-30 minutes when this test substrate is used (Washabau *et al* 1986b, Muir *et al* 1994, Papasoul *et al* 1994). However, a carbohydrate containing solid meal, that is well assimilated by healthy dogs and administered at commonly fed amounts (ie., 50% maintenance energy requirements), would be a more physiological test substrate. The main disadvantage with using such a substrate, is that gastrointestinal transit of solid food is slower than liquids, which results in the need for a longer test period. When prescription diet d/d (Hill's Pet Products, Topeka, Kansas) is fed as a test meal to dogs, breath H₂ values commonly begin to rise around 4-8 hours and reach a maximum at 6-12 hours^a (Washabau *et al* 1986a&b). Therefore, the breath testing period should be extended to at least 12 hours when a solid test meal is fed. Despite the longer test period required, samples need only be collected at hourly intervals. This results in a similar total number of breath samples collected over the test period.

In defining significant small intestinal malabsorption, fasting breath H₂ values, peak (maximum) breath H₂ values, and areas under the curve (AUC) values of patients vs healthy controls are often compared. A certain value rise over baseline (determined from healthy control data) is also commonly employed as a cut-off value for diagnosing significant malabsorption.

^a Guilford WG: Department Veterinary Clinical Sciences, Massey University, New Zealand. Personal communication, 1995.

The sensitivity of the breath H_2 test can be improved by assessing breath H_2 concentrations over time (eg., AUCs) rather than relying solely on peak breath H_2 values (Kotler *et al* 1982, Kerlin *et al* 1984). The use of summing the breath H_2 fractions measured over the test period (cumulative breath H_2) may be a useful and simple alternative to calculating AUCs in clinical practice. Because individual variation in breath H_2 excretion is great, reference ranges need to be established in healthy controls before wide use of breath H_2 testing in the investigation of small intestinal malabsorption is employed in animals. Given that different food products vary in nutrient profiles, nutrient source and food structure, differences in OCTT and the amount of H_2 produced are likely to occur when different solid meals are used. This prevents the use of reference ranges established for one food product being used for alternative test meals.

Other applications of the breath H_2 test in diseased individuals have included the investigation of the pathophysiology of symptoms and clinical signs in functional bowel disease, evaluation of the response to treatment of certain gastrointestinal diseases, and determining the appropriateness of certain foods for carbohydrate intolerant patients. People with functional bowel disease have been found to produce similar amounts of breath H_2 in response to test substrates as healthy controls, despite having clinical signs suggestive of carbohydrate intolerance (Saltzberg *et al* 1988, Fernandez *et al* 1993). This has led to the conclusion that these subjects have an increased perception of/or sensitivity to gas production rather than an absolute increase in the amount of intestinal gas produced. In evaluating the response to treatment of certain gastrointestinal disorders, both human and canine subjects with EPI have shown decreased carbohydrate malabsorption after the administration of pancreatic enzyme supplements (Mackie *et al* 1981, Kerlin *et al* 1984, Washabau *et al* 1986b). Two cats with inflammatory bowel disease have also been shown to produce normal breath H_2 values after treatment with oral prednisone and metranidazole (Papasouliotis *et al* 1994), although it is possible that the drugs administered interfered with breath H_2 production. Several breath H_2 studies of lactase deficient children have investigated the appropriateness of feeding various lactose containing products. Yoghurt which contains active bacterial cultures has been shown to be assimilated to a greater extent, and tolerated better than milk or pasteurised yoghurt (Shermak *et al* 1995). In addition, the chronic ingestion of low doses of lactose has been shown to be well tolerated in lactase deficient individuals due to adaptation of the colon to prolonged lactose malabsorption (Hertzler and Savaiano 1996).

Investigation of carbohydrate assimilation in healthy individuals

Since its development, the breath H_2 test has also been applied to the investigation of carbohydrate malabsorption and gas producing ability of various dietary carbohydrates in

healthy individuals. Work in this area has several implications in man, particularly in relation to the management of gastrointestinal disorders, diabetes mellitus, flatulence, obesity, and the prevention of colon cancer. Many of these areas are also relevant to canine and feline health, and thus the opportunity exists to utilise this test further in veterinary medicine.

Breath H₂ testing in healthy people has identified several important characteristics of different dietary carbohydrates. Anderson *et al* (1981) showed rice and gluten free flours to have superior digestibility to all-purpose wheat flour and pasta, and suggested that the use of these flours in the management of gastrointestinal disease may play an important role in reducing clinical signs. Further evidence that rice flour is more completely assimilated than other flours in healthy people and dogs exist (Levine and Levitt 1981, Levitt *et al* 1987, Washabau *et al* 1986a). Certain sugars (eg., fructose, sorbitol) have been shown to be malabsorbed by a large percentage of individuals, at doses commonly present in foods and drinks (Ravich *et al* 1983, Fernandez *et al* 1993). These findings have implicated these sugars in the development of gastrointestinal symptoms, particularly in functional bowel disease. The ability to increase the assimilation of these sugars by concurrent consumption of glucose, amino acids, or lipids has also been documented by breath H₂ tests (Beaugerie *et al* 1996, Hoekstra and Vandenaker 1996). This increased assimilation is thought to occur by delaying gastric emptying.

The consumption of beans is associated with dramatically increased breath H₂ values in comparison to other carbohydrates, confirming the high gas producing ability of these carbohydrates and their role in the production of flatus (Calloway 1966, Levine and Levitt 1981, Levitt *et al* 1987). Foods containing various concentrations of resistant starch have also been investigated for their gas producing ability *in vivo* using breath H₂ analysis (Olesen *et al* 1992). Some types of resistant starch were shown to be poor H₂ producers. The presence of fibre in carbohydrate sources has been shown to increase breath H₂ production significantly, presumably by decreasing starch assimilation, but also possibly because of fermentation of the fibre itself (Levitt *et al* 1987). Breath H₂ testing has also been applied to dogs to demonstrate if the addition of fibres to a canned diet decreases post prandial hyperglycaemia without significantly altering the OCTT (Papasouliotis *et al* 1993b). The addition of different fibres to diets has also been shown to affect breath H₂ measurements (Davenport *et al* 1993).

Breath H₂ has also been used to investigate the effects of food processing on carbohydrate assimilation. Casiraghi *et al* (1992) used breath H₂ to compare the assimilation of rice processed in three different ways, while Cloarec *et al* (1988) utilised breath H₂ to investigate the effect of cooking time on pasta assimilation.

Some of the nutritional applications of the breath H₂ test are no longer recommended because they relied on assumptions that have been subsequently shown to be false. For example, the breath H₂ test (using lactulose as an internal standard) has been used in the past as an alternative method to intubation studies and ileostomy studies for quantitating malabsorbed carbohydrate (Bond and Levitt 1972, Levitt *et al* 1987, Flourie *et al* 1988a). Several problems were identified using this method of estimating starch malabsorption (eg., different fermentabilities of various carbohydrates) and results were found to be inaccurate (Cummings and Englyst 1991, McBurney 1991) (see “Variables and limitations of the breath H₂ measurements” for more details).

Variables and Limitations of Breath Hydrogen Measurements

Breath H₂ concentrations can be affected by a variety of variables which may compromise the value of the breath H₂ test. Because these variables may jeopardise the accuracy of this test and influence the interpretation of the breath H₂ readings, it is important to be aware that such variables exist and to minimise their influence. The factors affecting breath H₂ concentrations can be broadly classified into gastrointestinal and extraintestinal factors.

Gastrointestinal factors

Many gastrointestinal factors play an important role in determining the amount of H₂ that will be produced and excreted in the breath. The most important gastrointestinal factor affecting breath H₂ excretion relates to the numbers, activities and composition of microbes in the colon.

Intestinal microbial population

The production and fate of H₂ gas in the colon is largely dependent on the microbial population present. The numbers and metabolic activities of H₂ producing microbes, as well as the numbers and metabolic activities of H₂ consuming bacteria (methanogens, sulfate reducing bacteria, acetate forming bacteria) are critical in determining the “net” amount of H₂ that will be available for excretion in the breath and flatus (Strocchi and Levitt 1992a & b). People that do not produce H₂ in the breath (H₂ non-producers) and people that produce significant amounts of breath methane (CH₄ producers) are thought to decrease the diagnostic accuracy of the H₂ breath test because of their inferior net H₂ producing ability (Volgelsang *et al* 1988, Cloarec *et al* 1990).

“Hydrogen non-producers” or “poor H₂ producers” are individuals that do not

demonstrate a significant rise in breath H_2 excretion after the ingestion of a non-absorbable carbohydrate (eg., lactulose). The prevalence of H_2 non-producers is estimated to be between 0-27% of the human population (Levitt and Donaldson 1970, Bond *et al* 1975, Gilat *et al* 1978, Bjorneklett and Jenssen 1982, Davidson and Robb 1985, Vogelsang *et al* 1988, Saltzberg *et al* 1988, Cloarec *et al* 1990). The prevalence varies between studies mainly because of different diagnostic criteria applied to define a negative or positive result. When "failure to produce H_2 " is defined as an inability to elevate breath H_2 concentrations 20 ppm or more above baseline after a 10-20 gram oral lactulose load, the prevalence of H_2 non-producers is high (Saltzberg *et al* 1988). This 20 ppm rise is the same value that is commonly used for diagnosing clinically significant carbohydrate malabsorption (Metz *et al* 1975). In comparison, the prevalence of H_2 non-producers is extremely low (<5%) when defined as an individual unable to produce any recognisable increase in breath H_2 excretion (Levitt and Donaldson 1970, Bond *et al* 1975, Strocchi *et al* 1993). Hydrogen "non-producers" may be capable of producing significant amounts of H_2 in response to carbohydrate substrates other than lactulose (Mishkin *et al* 1993, Ladas *et al* 1993), and faecal incubation studies have paradoxically shown that the H_2 producing ability of H_2 "non-producers" may be similar to that of H_2 producers (Bjorneklett and Jenssen 1982). This suggests that other factors apart from an inherently poor H_2 producing ability is responsible for the low breath H_2 excretion. Factors which are known to predispose to the "non H_2 producing" state include: oral antibiotic therapy (Davidson and Robb 1985), an acidic colonic pH (Vogelsang *et al* 1988), and individuals who produce high concentrations of CH_4 in the breath (Bjorneklett and Jenssen 1982, Cloarec *et al* 1990).

Evidence that H_2 "non-producers" are capable of producing significant amounts of H_2 , but display increased H_2 consumption has been provided by Strocchi and Levitt (1992a). These investigators demonstrated that H_2 consumption by faecal bacteria varies directly with H_2 tension, and occurs to a greater degree in faeces containing methanogens. Faeces from low H_2 producers displayed rapid methanogenesis, or were considered to have increased H_2 consumption due to high faecal H_2 tensions created from decreased faecal mixing/stirring. Methanogenesis appears to be the predominate pathway of H_2 consumption in man, however, other pathways for H_2 disposal (sulphate reduction, acetogenesis) also exists (Lajoie *et al* 1988, Gibson *et al* 1990, Strocchi *et al* 1993b). Methanogenic bacteria are efficient H_2 consumers (Strocchi and Levitt 1992a, Strocchi *et al* 1993b), and therefore CH_4 producing individuals often excrete less H_2 in comparison to non- CH_4 producers (Bjorneklett and Jenssen 1982, Cloarec *et al* 1990, Christl *et al* 1992). Some investigators have advised performing both H_2 and CH_4 breath tests concurrently, to adequately assess the degree of carbohydrate fermentation and enhance the accuracy of the breath H_2 test (Bjorneklett and Jenssen 1982, Corazza *et al* 1994).

The prevalence of CH₄ producers in the adult population is reported to be 24-65% (Corazza *et al* 1994). The incidence varies between ethnic groups and has a strong familial association (Bond *et al* 1971, Pitt *et al* 1980). Methanogenic bacteria are thought to reside mainly in the left colon where the pH is higher and the environment more favourable for their growth (Flourie *et al* 1990). In comparison to H₂, CH₄ excretion in the breath remains relatively constant throughout the day (Calloway and Murphy 1968), has much higher fasting values (Corazza *et al* 1994), and requires larger doses of lactulose to stimulate increased excretion (Flourie *et al* 1990). These differences are probably related to the more distal anatomical location of methanogens within the gastrointestinal tract. It is interesting to note that substrates metabolised over a large area of the colon (eg., slowly fermentable substrates) are more likely to produce less H₂ than substrates which are rapidly metabolised in the proximal colon. This occurs because slowly fermentable substrates interact with greater quantities of methanogens or other H₂ consuming bacteria (Strocchi and Levitt 1992a).

In comparison to man, the consumption of intestinally derived H₂ by methanogenic bacteria appears to be less important in dogs and cats. The prevalence of CH₄ producers in carnivores appears to be low, as reported by several investigators (Richards and Steggerdia 1966, Miller and Wolin 1986, Schlesinger *et al* 1993). However, *in vitro* fermentation of canine ileal chyme has demonstrated that the dog is capable of producing at least small amounts of CH₄ (Grubler *et al* 1990). Although CH₄ production in dogs and cats appears to be much less than in humans, it is probable that H₂ consuming bacteria other than methanogens (sulfate reducing bacteria, acetate forming bacteria) play a significant role in altering net H₂ production in the canine and feline gastrointestinal tracts.

Oropharyngeal microbes & the gastro-ileal reflex

The phenomenon of an early breath H₂ rise occurring within the first 30 minutes following the ingestion of a carbohydrate test meal has been recognised in a large percentage of people undergoing breath H₂ tests (Calloway and Murphy 1968, Read *et al* 1985, Mastropaolo and Rees 1987). This rise precedes the larger breath H₂ rise due to malabsorbed carbohydrates entering the colon, and may be due to fermentation of food by oropharyngeal microbes and/or increased ileal output of fermentable substrates present in the small intestine (remnants of a previous meal or gastrointestinal secretions) which occurs with eating (gastro-ileal reflex) (Thompson *et al* 1985, *et al* 1986, Read *et al* 1985). The evidence for oropharyngeal fermentation of food substrates causing an early breath H₂ rise is strong (Thomson *et al* 1985, *et al* 1986). Increased breath H₂ excretion has been demonstrated following a sucrose solution mouthwash, meal ingestion, and sham feeding. However, when tooth brushing and a 1%

chlorhexidine mouthwash preceded the test, the early increase in breath H₂ was reduced or abolished. This early breath H₂ rise can also be greatly reduced by delivering the test meal by stomach tube. Read *et al* (1985) used patients with ileostomies to show that increased ileal output of intestinal contents (mainly bile stained mucus) occurred after meal ingestion, and that this phenomenon may also contribute to the early breath H₂ rise. These authors also observed that the initial breath H₂ peak tended to be much larger if the subject had eaten a meal containing unabsorbable carbohydrates late on the previous evening. This suggested that fermentable substrates entering the colon by the gastro-ileal reflex contain remnants of the previous meal.

Early breath H₂ rises may interfere with the interpretation of breath H₂ results, particularly in determining OCTT and in testing for SIBO. These early rises can be effectively eliminated if attention is paid to oral hygiene and if meals prior to the breath H₂ test period are low in indigestible carbohydrates (Cloarec *et al* 1990). The phenomenon of an early breath H₂ rise has not yet been demonstrated in dogs. However, breath testing in this species commonly utilises hourly sampling intervals, over which time an early postprandial H₂ rise could be easily missed.

Rate of colonic entry of fermentable substrates

Apart from the amount and type of carbohydrate reaching the colon, the rate at which carbohydrates arrive at the colon may effect the timing and the magnitude of the breath H₂ response. Read *et al* (1985) demonstrated that a rapid colonic infusion of lactulose produced a greater H₂ response than a slow infusion of the same dose. This may indicate that rapid small intestinal transit times can result in greater breath H₂ responses, if colonic entry rate is also increased. In the above study, however, the difference in infusion rates was large (sevenfold) and total breath H₂ production post infusion was not compared. In a subsequent study (Rumessen *et al* 1989), different orocolic transit times were created by the administration of motility modifiers. This study was unable to confirm the previous findings, as no significant differences in breath H₂ values were found between groups.

Colonic motility

It is possible that colonic motility influences breath H₂ excretion. Rapid colonic transit times could reduce the breath H₂ response to a fermentable substrate by limiting the time available for fermentation or by limiting the time available for colonic buffering (see "Colonic pH" below). It is also possible that decreased colonic activity may impair faecal stirring, thereby

decreasing net H₂ production by favouring H₂ consumption (see "Intestinal microbial population" above).

Colonic pH

The administration of lactulose has been shown to produce marked acidification of the proximal colon, presumably due to bacterial fermentation with acid production at this site (Bown *et al* 1974). Chronic administration of lactulose to healthy individuals may dramatically reduce the breath H₂ response to a lactulose load (Perman *et al* 1981a, Vogelsang *et al* 1988, Florent *et al* 1985). Perman *et al* (1981a) was the first to demonstrate this effect, and showed that mean breath H₂ excretion could be reduced by more than 70% over a four hour testing period following a 20g lactulose load. Studies were also undertaken using *in vitro* faecal incubation techniques, and maximal production of H₂ gas and maximum glucose disappearance was shown to occur at a faecal pH of 7.0-7.45. Reduction of faecal pH to 6.2 and 5.5 resulted in significantly less H₂ production, and a pH of 5.0 virtually eliminated all H₂ evolution. The authors concluded that bacterial metabolic activity was inhibited at low pH (rather than a change bacterial composition), as reversal of the inhibition of H₂ production could be achieved by neutralising the faecal incubates.

In contrast to the above theory of acidic inhibition of fermentation, Florent *et al* (1985) provided evidence that the reduction of breath H₂ values following a chronic lactulose load is due to bacterial adaptation and more efficient carbohydrate metabolism. A lack of faecal sugar excretion and the absence of a reduction of faecal pH after 8 days of lactulose ingestion did not support inhibition of fermentation. However, significant increase in β -galactosidase activity in the faeces, significant changes in caecal contents (decreased lactulose concentrations, lower pH drop, increased volatile fatty acids and lactic acid concentrations), and elevated ¹⁴CO₂ concentrations in the breath were all supportive of changes in the bacterial metabolism of lactulose induced over the 8 day period. It is possible that a reduced caecal pH favours the growth of lactic acid producing bacteria (eg., *Lactobacillus*, *Bifidobacterium*, *Eubacterium*) and is responsible for a change in the end products of fermentation.

Colonic perfusion

Breath H₂ concentrations have been shown to be dramatically reduced in hypovolaemic dogs and in dogs in which the mesenteric blood flow has been surgically occluded (Perman *et al* 1981b). Breath H₂ studies were performed on anaesthetised dogs in which isolated ileal segments were insufflated with 100% H₂ gas over 40 minute periods in (i) control dogs, (ii) dogs in which 10% of the estimated blood volume was removed on three successive occasions by

phlebotomy, and (iii) in dogs after clamping of mesenteric vessels to the isolated ileal segment. The induction of hypovolaemia resulted in decreased breath H₂ excretion (77, 66 & 35 % of baseline respectively) which paralleled the decrease in blood pressure. However, the drop in breath H₂ excretion in comparison to baseline was only significant following the 30% loss of blood volume. Although hypovolemia may have resulted in decreased breath H₂ values by several mechanisms (eg., decreased intestinal H₂ absorption due to decreased mesenteric blood flow; increased minute ventilation, reduced pulmonary blood flow and H₂ diffusion), the fact that ventilation was controlled during the procedure, and that mesenteric vessel clamping to the ileal segment produced a very similar decline in breath H₂ excretion (39% of baseline) supported that hypovolemia probably resulted in decreased intestinal perfusion thereby reducing breath H₂ excretion

Diarrhoea

Solomons *et al* (1979) reported that children undergoing breath H₂ tests during episodes of diarrhoea may display poor rises in breath H₂ values. This observation was formed from clinical experience, and a study where 10 children with acute gastroenteritis were shown to have significantly lower breath H₂ measurements than 5 children without diarrhoea. However, 3 of the 10 diarrhoeic children were receiving antibiotics prior to the breath H₂ test. In contrast to the above findings, another breath H₂ study on children with diarrhoea (Davidson and Robb 1985) recorded breath H₂ rises of expected magnitude. It is feasible, however, that breath H₂ testing during diarrhoea could be affected by numerous factors including: qualitative or quantitative changes in the gastrointestinal microflora, gastrointestinal transit changes (less time available for fermentation, more H₂ excreted as flatus), and other possible changes in the colonic microenvironment (eg., colonic acidification).

Extraintestinal factors

A number of extraintestinal factors also influence exhaled breath H₂ measurements. The most important of these is the amount and type (fermentability characteristics) of food used in the test. Given that there is generally more control over extraintestinal variables on breath H₂ excretion than gastrointestinal ones, it is important to be aware that these factors discussed below exist, and to minimise them during the test procedure.

Amount & type of food consumed

It has been demonstrated on a number of occasions that a linear correlation exists between the amount of breath H₂ excreted and amount of carbohydrate malabsorbed (Bond and

Levitt 1972, Solomons and Viteri 1978, Fritz *et al* 1985, Washabau *et al* 1986a, Rumessen *et al* 1990). Most of the evidence for the linearity of this relationship has been determined by increasing lactulose doses. However, there is also some evidence that malabsorption of other carbohydrates results in a linear increase in breath H₂ values. Increasing amounts of potato starch fed to people with ileostomies showed a linear relationship between starch input and output (Chapman *et al* 1985). One hundred grams of different carbohydrate sources fed to subjects resulted in approximately twice as much breath H₂ as the same carbohydrate sources fed in 50g amounts (Levitt *et al* 1987), and caecal infusion of increasing amounts of raw wheat starch also caused what appeared to be a linear increase in breath H₂ measurements (Flourie *et al* 1988a). These results show that the breath H₂ test is a semiquantitative one, as it gives information regarding the amount of carbohydrate malabsorbed. Despite these findings, the ability of the breath H₂ test to semiquantitatively assess the malabsorption of complex carbohydrates has recently been questioned (Flourie *et al* 1988b, Nordgaard *et al* 1995). Flourie *et al* (1988b) demonstrated similar breath H₂ excretion in healthy people fed diets containing 100 and 300 grams of starch. Similarly, Nordgaard *et al* (1995) found that short-bowel patients fed isocaloric diets with different carbohydrate contents (20% vs 60%) did not have significantly different peak breath H₂ responses or AUCs. Although Flourie's study demonstrated a significant difference in the amount of starch recovered from the terminal ileum via aspiration after feeding the two different diets, Nordgaard's study did not directly demonstrate a difference in carbohydrate malabsorption between the diets used. It is also possible that the 6 day period where the test diet was fed prior to breath testing in Flourie's study may have resulted in colonic adaption to the increased carbohydrate load (see "Frequency/regularity of food ingestion" below).

Carbohydrates are commonly regarded as the sole substrate for microbial production of H₂, but it has been demonstrated on several occasions that proteins may also be fermented with H₂ produced (Calloway *et al* 1966, Carter *et al* 1981, Perman and Modler 1982, Thompson *et al* 1986, Zentek *et al* 1993). The addition of amino acids (Calloway *et al* 1966) and glycoproteins (Perman and Modler 1982) to human faecal homogenates results in H₂ production, as does the addition of bovine serum albumin and casein hydrolysate to rat faecal homogenates or rat digestive systems (Carter *et al* 1981). In addition, the consumption of a protein (beef) meal by healthy people (Thompson *et al* 1986) resulted in some H₂ evolution, while raw offal fed to dogs has been shown to cause significant breath H₂ excretion (Zentek *et al* 1993). Generally, the amount of H₂ produced from protein substrates is considerably less than H₂ produced from similar amounts of fermentable carbohydrates. These findings need to be

considered when mixed meals are fed, as the carbohydrate constituents of the meal may not be the only substrates contributing to H₂ production.

Originally it was believed that different carbohydrate substrates produced similar amounts of H₂ gas per gram fermented. This belief was founded on work which assessed the degree of H₂ production after the administration of equal volumes and concentrations of various sugars (Bond and Levitt 1972, Carter *et al* 1981, Perman *et al* 1981). However, it is now known that different carbohydrate substrates may produce variable amounts of H₂ gas (Levitt *et al* 1987, McBurney and Thompson 1989, Christl *et al* 1992, Olsen *et al* 1992, Livesey *et al* 1993, Grubler *et al* 1990). On a weight-for-weight basis, fibres have been shown to produce less H₂ than sugars and starch (Levitt *et al* 1987), banana starch and pectin to produce less H₂ than lactulose (Christl *et al* 1992), and different fibres have been shown to produce variable amounts of H₂ gas (McBurney and Thompson 1989). The H₂ producing ability of a particular carbohydrate substrate is probably determined by a number of factors which determine its fermentability eg., physical form, solubility, rate of depolymerisation, and chemical composition. Rapidly fermentable substrates may result in decreased colonic H₂ absorption in comparison to slowly fermentable substrates by producing large gas volumes that enhances colonic transit and promote rectal gas excretion. However, they are more likely to produce greater net H₂ volumes, as entry to the more distal colon where H₂ consumption predominates is less likely (Hammer 1993, Strocchi and Levitt 1992a). The variation of H₂ production with carbohydrate type explains why quantitative estimates of carbohydrate malabsorption using lactulose as an internal standard are often inaccurate (Cummings and Englyst 1990, McBurney 1991, Cummings and Englyst 1991).

Frequency/regularity of food ingestion

Regular ingestion of a H₂ producing substrate may result in decreased H₂ production over time, presumably as colonic bacteria adapt and metabolise the substrate more efficiently to a more reduced end product (eg., volatile fatty acids and lactate). Hertzler and Savaiano (1996) supplemented 20 lactose intolerant people with increasing doses of lactose or dextrose over a 10 day period. They found breath H₂ concentrations to be significantly reduced after a lactose breath H₂ test on the 11th day of lactose supplementation. Symptoms of lactose intolerance were also significantly reduced. Increased concentrations of faecal β -galactosidase after regular lactose ingestion has been shown to occur within 48 hours of lactose ingestion, and this presumably allows for more rapid lactose hydrolysis and efficient lactose catabolism. The reduced production of H₂ is probably related to the proliferation of lactose fermenting, non-H₂

producing bacteria. This is the same mechanism that has been proposed to explain the effect of acidifying the colon by chronic lactulose ingestion (see “Colonic pH” above).

Dilution of H₂ by collection technique

Collection of mixed expired air via the anaesthetic face mask technique, as commonly used in human paediatric and veterinary patients, invariably results in dilution of H₂ gas due to dead space (anatomical dead space and dead space in the collection apparatus) (see “Collection techniques and sample storage” for details).

Dead space dilution introduced by the anaesthetic face mask technique is not necessarily a major problem in obtaining reliable data to predict carbohydrate malabsorption (Holland *et al* 1986). Given that measures are taken to minimise and keep dilution rates constant (eg., use of a collection apparatus of appropriate size for the animal undergoing testing, adequate flushing of the collection apparatus with expired air before sample collection begins, and minimising factors affecting ventilation rates), dead space dilution is unlikely to impair the diagnostic accuracy of the H₂ breath test.

Ventilation effects

An important error in the breath H₂ technique as applied to human paediatric and veterinary medicine relates to poor control over ventilation rates. Breath samples collected from children who fall asleep during tests show marked (over 2 fold) increases in breath H₂ concentrations. Hypoventilation due to a fall in minute volume and a rise in PCO₂, and changes in colonic motility, are the postulated causes of increased breath H₂ concentrations measured during sleep. Hypoventilation is thought to be the main cause of increased breath H₂ concentrations, as great fluctuations were observed between sleep and wake cycles (Solomons and Viteri 1976, *et al* 1978, Metz and Jenkins 1977). Exercise has also been shown to affect breath H₂ concentrations dramatically. In one study, 5 minutes of step-ups caused breath H₂ concentrations to fall to zero in all subjects, and took up to 30 minutes to recover to pre exercise values (Payne *et al* 1983). In another study 2 minutes of slow steady walking significantly decreased breath H₂ concentrations (by approximately 50%) and returned to pre exercise values within 5 minutes (Thompson *et al* 1985). Hyperventilation is the likely cause of decreased breath H₂ concentrations after exercise, as it has been shown that breath H₂ concentrations vary inversely with minute ventilation (Perman *et al* 1985). In human subjects, the washout effect of alveolar H₂ due to hyperventilation can be decreased by delaying sampling for at least 5 minutes after any exercise and by training patients in breath collection techniques. Unfortunately with veterinary subjects, control over excitement and exercise that leads to hyperventilation is

difficult. However, restricting exercise during test periods and calming animals for 5 minutes prior to breath sampling may help to minimise hyperventilation.

Antibiotic therapy

Antibiotic therapy has been shown to alter the breath H_2 response to carbohydrates in humans (Murphy and Calloway 1972, Solomons and Viteri 1978, Gilat *et al* 1978, Strocchi *et al* 1993), rats (Levitt *et al* 1974), dogs (Richards and Steggerdia 1966, *et al* 1968), cats (Muir *et al* 1996) and calves (Holland *et al* 1986). In addition, antibiotics have also been shown to reduce breath H_2 values after a protein meal in dogs (Zentek *et al* 1993). Various types of antibiotics (eg., neomycin, sulfathalidine, trimethoprim sulphonamides, ampicillin, oxytetracycline, erythromycin) may cause marked depression of the amount of H_2 exhaled in the breath (Richards and Steggerdia 1966, Murphy and Calloway 1972, Solomon *et al* 1978, Gilat *et al* 1978). The decrease in breath H_2 excretion can occur after as little as two days of treatment (Murphy and Calloway 1972), and a return to higher H_2 values has been detected two weeks following the completion of the antibiotic course (Gilat *et al* 1978). Both *in vivo* and *in vitro* tests in dogs utilising a pork/bean homogenate added to isolated intestinal segments and anaerobic intestinal cultures, have demonstrated dramatically reduced gas production following antibiotic treatment (Richards *et al* 1968). Antibiotic suppression of H_2 producing bacteria is presumably the main reason for the depressed breath H_2 response.

Antibiotic therapy has also been associated with increased breath H_2 excretion in comparison to untreated controls (Murphy and Calloway 1972, Levitt *et al* 1974, Holland *et al* 1986, Strocchi *et al* 1993). It is possible that net H_2 production may increase if the H_2 consuming bacteria are primarily inhibited by antibiotic therapy, or if non- H_2 producing bacteria are primarily inhibited therefore allowing an increase in H_2 producing microbes. This theory is supported by the findings of Murphy and Calloway (1972) where marked increases in H_2 in breath and flatus were associated with decreased CH_4 values during succinylsulfathiazole treatment, and by Levitt *et al* (1974) who demonstrated that fixed doses of glucose instilled into the caecum of rats produced more H_2 after neomycin treatment than was measured before treatment. Alternatively, antibiotics may increase H_2 production by inducing intestinal damage and promoting carbohydrate malabsorption (Holland *et al* 1986). Increased breath H_2 excretion after oral oxytetracycline and metronidazole therapy has been reported in cats tested with xylose, however it is unclear whether this effect was due to microbial changes or increased small intestinal malabsorption of xylose (Muir *et al* 1996).

Regardless of the mechanism, it is obvious that antibiotic therapy may potentially alter any animal's H_2 production, and therefore should be stopped for at least 2 weeks prior to breath

H₂ testing. Results of breath H₂ tests on animals being treated with antibiotics need to be interpreted with caution.

Colonoscopy/enemas

Mechanical cleansing of the colon in preparation for colonoscopy using a combination of laxatives and enemas has been shown to cause a marked decrease in breath H₂ concentrations in response to oral lactulose (Gilat *et al* 1978). Cleansing of the colon may alter the colonic luminal environment and decrease bacterial numbers, both of which can potentially alter intestinal H₂ production and excretion. Given the above findings, breath H₂ testing should be avoided immediately after colonoscopy procedures.

Anxiety

There is evidence in humans that fluctuations in breath H₂ excretion may occur in response to anxiety. Studies undertaken by Calloway and Murphy (1968) showed that breath H₂ tests performed in a crowded, busy laboratory resulted in elevated breath H₂ values in comparison to the same test performed in a quiet room with comfortable furnishings. In addition, a ten fold increase in breath H₂ concentrations was seen in an individual informed he would need to repeat an unpleasant experience. How psychological states affect breath H₂ excretion is unclear.

Age

Neonates appear to excrete greater amounts of breath H₂ than older children and adults which makes diagnosis of carbohydrate malabsorption difficult by this method in this age group (Maclean and Fink 1980, Stevenson *et al* 1982, Millar *et al* 1992). Breath H₂ values in neonates are generally highest in the first month of life with significantly lower (but still elevated in comparison to older children) values in the second and third months (Millar *et al* 1992). Hydrogen production is thought to be higher in infants due to immaturity of carbohydrate absorption. Other gastrointestinal factors such as gastrointestinal perfusion, motility, microflora, and luminal pH may also differ in comparison to older subjects. Another peculiarity in breath H₂ profiles of infants is a lack of postprandial breath H₂ peaks or typical patterns of H₂ production seen in adults (Stevenson *et al* 1982, Millar *et al* 1992). Several factors may contribute to this difference, including a lack of adequate fasting prior to breath testing, increased frequency of feeding, prolonged gastrointestinal transit or significant H₂ production from endogenous carbohydrate sources.

Elderly individuals have also been shown to exhale more H₂ in comparison to younger adults (Saltzberg *et al* 1988). Breath H₂ testing following lactulose administration in 18 elderly subjects (mean age 76 years) and 34 younger adults (mean age 32 years) showed significantly increased breath H₂ concentrations in the older age group. Several gastrointestinal factors could contribute to differences between these two age groups, such as differences in bacterial populations and differences in gastrointestinal transit times.

Cigarette smoking

One problem that is encountered with breath H₂ testing in human medicine, and one that is unlikely to pose a problem in veterinary patients is the effect of cigarette smoking. Smoking is associated with marked breath H₂ rises which return to normal values soon after smoking ceases. The increased H₂ readings are due to a combination of H₂ production from tobacco combustion and carbon monoxide production; a gas which the electrochemical H₂ detector is partially responsive to (Thompson *et al* 1985).

References

- Anderson IH, Levine AS, Levitt MD: Incomplete absorption of the carbohydrate in all-purpose wheat flour. *New Eng J Med* 304:891-892, 1981.
- Ambrecht U, Jensen J, Eden S, Stockbrugger R: Assessment of oro-caecal transit time by means of a hydrogen (H₂) breath test as compared with a radiologic control method. *Scand J Gastroenterol* 21:669-677, 1986.
- Barr RG, Watkins JB, Perman JA: Mucosal function and breath hydrogen excretion: comparative studies in the clinical evaluation of children with nonspecific abdominal complaints. *Pediatrics* 68:526-533, 1981.
- Bartlett K, Dobson JV, Eastham E: A new method for the detection of hydrogen in breath and its application to acquired and inborn sugar malabsorption. *Clin Chim Acta* 108:189-194, 1980.
- Batt RM, Hall EJ: Chronic enteropathies in the dog. *J Sm Anim Pract* 30:3-12, 1989.
- Batt RM, Barnes A, Rutgers HC, *et al*: Relative IgA deficiency and small intestinal bacterial overgrowth in German Shepherd Dogs. *Res Vet Sci* 50:106-111, 1991.
- Beaugerie L, Lemann M, Jian R, *et al*: Effect of glucose and lipids on intestinal absorption of sorbitol- role of gastric emptying. *Neurogastroenterology Motility* 8:235-239, 1996.
- Bjorneklett A, Jenssen E: Measurement of pulmonary hydrogen (H₂) and H₂ diffusion from the small bowel and the colon. *Scand J Gastroenterol* 15:817-823, 1980.

- Bjorneklett A, Jenssen E: Relationships between hydrogen (H₂) and methane (CH₄) production in man. *Scand J Gastroenterol* 17:985-992, 1982.
- Bond JH, Engel RR, Levitt MD: Factors influencing pulmonary methane excretion in man. *J Exp Med* 133:572-588, 1971.
- Bond JH, Levitt MD: Use of pulmonary hydrogen measurements to quantitate carbohydrate absorption. *J Clin Invest* 51:1219-1225, 1972.
- Bond JH, Levitt MD, Prentiss R: Investigation of small bowel transit time in man utilising pulmonary hydrogen measurements. *J Lab Clin Med* 85:546-555, 1975.
- Bown RL, Gibson JA, Sladen GE, *et al*: Effects of lactulose and other laxatives on ileal and colonic pH as measured by a radiotelemetry device. *Gut* 15:999-1004, 1974.
- Bracher V, Steiger R, Huser S: Preliminary results of a combined xylose absorption/hydrogen exhalation test in horses. *Schweizer Archiv fur Tierheilkunde* 137:297-305, 1995.
- Braden B, Braden CP, Klutz M, Lembcke B: Analysis of breath hydrogen (H₂) in diagnosis of gastrointestinal function: validation of a pocket breath H₂ test analyzer. *Z Gastroent* 31:242-245, 1993.
- Brown NJ, Rumsey RDE, Read NW: Adaptation of hydrogen analysis to measure stomach to caecum transit time in the rat. *Gut* 28:849-854, 1987.
- Calloway DH, Colasito DJ, Mathews RD: Gases produced by human intestinal microflora. *Nature* 212:1238-1239, 1966a.
- Calloway DH: Respiratory hydrogen and methane as affected by consumption of gas-forming foods. *Gastroenterology* 51:383-389, 1966b.
- Calloway DH, Murphy EL: The use of expired air to measure intestinal gas formation. *Ann New York Acad Sc* 150:82-95, 1968.
- Calloway DH, Murphy EL, Bauer D: Determination of lactose intolerance by breath analysis. *Am J Dig Dis* 14:811-815, 1969.
- Cann PA, Read NW, Brown C, *et al*: Irritable bowel syndrome: relationship of disorders in the transit of a single solid meal to symptom patterns. *Gut* 24:405-411, 1983.
- Caride VJ, Prokop EK, Troncale FJ, *et al*: Scintigraphic determination of small intestinal transit time: comparison with the hydrogen breath technique. *Gastroenterology* 86:714-720, 1984.
- Carter EA, Bloch KJ, Cohen S, *et al*: Use of hydrogen gas analysis to assess intestinal absorption: studies in normal rats and in rats infected with the nematode, *Nippostrongylus brasiliensis*. *Gastroenterology* 81:1091-1097, 1981.
- Casiraghi MC, Brighenti F, Pellegrini N, *et al*: Effect of processing on rice starch digestibility evaluated by *in vivo* and *in vitro* methods. *J Cereal Sci* 17:147-156, 1993.
- Chapman RW, Sillery JK, Graham MM, Saunders DR: Absorption of starch by healthy ileostomates: effect of transit time and of carbohydrate load. *Am J Clin Nutr* 41:1244-1248, 1985.

- Christl SU, Murgatroyd PR, Gibson GR, Cummings JH: Production, metabolism, and excretion of hydrogen in the large intestine. *Gastroenterology* 102:1269-1277, 1992.
- Cloarec D, Bornet F, Gouilloud S, *et al*: Breath hydrogen response to lactulose in healthy subjects: relationship to methane producing status. *Gut* 31:300-304, 1990.
- Cloarec D, Bornet F, Barry JL, *et al*: Starch malabsorption in healthy subjects. *Gastroenterology* 94:A72, 1988.
- Corazza GR, Strocchi A, Gasbarrini G: Fasting breath hydrogen in celiac disease. *Gastroenterology* 93:53-58, 1987.
- Corazza GR, Menozzi MG, Strocchi A, *et al*: The diagnosis of small bowel bacterial overgrowth. *Gastroenterology* 98:302-309, 1990.
- Corazza GR, Benati G, Strocchi A, *et al*: The possible role of breath methane measurement in detecting carbohydrate malabsorption. *J Lab Clin Med* 124:695-700, 1994.
- Cummings JH, Englyst HN: Breath hydrogen (H₂) may not be a reliable way of quantitating starch fermentation. *Gastroenterology* 98:A166, 1990.
- Cummings JH, Englyst HN: Measurement of starch fermentation in the human large intestine. *Can J Physiol Pharmacol* 69:121-129, 1991.
- Davenport DJ, Salter K, Twedt DC, Gross KL: Dietary fibre effects on breath hydrogen measurements in dogs. *Waltham Symposium on the Nutrition of Companion Animals*, Adelaide, South Australia, September 1993, pp 83.
- Davenport DJ, Ching RJW, Hunt JH, *et al*: The effect of dietary levels of folate and cobalamin on the serum concentration of folate and cobalamin in the dog. *J Nutr* 124:2559S-2562S, 1994.
- Davenport DJ: Small intestinal bacterial overgrowth- need for a new definition? *Proc ACVIM* 14:346-347, 1996.
- Davidson GP, Robb TA: Value of breath hydrogen analysis in management of diarrheal illness in childhood: comparison with duodenal biopsy. *J Pediatr Gastroenterol Nutr* 4:381-387, 1985.
- de Boissieu D, Chaussain M, Badoual J, *et al*: Small-bowel bacterial overgrowth in children with chronic diarrhea, abdominal pain, or both. *J Pediatr* 128:203-207, 1996.
- Di Lorenzo C, Dooley CP, Valenzuela JE: Role of fasting gastrointestinal motility in the variability of gastrointestinal transit time assessed by hydrogen breath test. *Gut* 32:1127-1130, 1991.
- Duan LP, Braden B, Clement T, *et al*: Clinical evaluation of a miniturized desktop breath hydrogen analyzer. *Z Gastroent* 32:575-578, 1994.
- Ellis CJ, Kneip JM, Levitt MD: Storage of breath samples for H₂ analyses. *Gastroenterology* 94:822-824, 1988.
- Fernandez-Banares F, Esteve-Pardo M, de Leon R, *et al*: Sugar malabsorption in functional bowel disease: clinical implications. *Am J Gastroenterol* 88:2044-2050, 1993.

- Florent C, Flourie B, Leblond A, *et al*: Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an *in vivo* study). *J Clin Invest* 75:608-613, 1985.
- Flourie B, Florent C, Etanchaud F, *et al*: Starch absorption by healthy man evaluated by lactulose hydrogen breath test. *Am J Clin Nutr* 47:61-66, 1988a.
- Flourie B, Leblond A, Florent C, *et al*: Starch malabsorption and breath gas excretion in healthy humans consuming low- and high-starch diets. *Gastroenterology* 95:356-363, 1988b.
- Flourie B, Etanchaud F, Florent C, *et al*: Comparative study of hydrogen and methane production in the human colon using caecal and faecal homogenates. *Gut* 31:684-685, 1990.
- Fritz M, Siebert G, Kasper H: Dose dependence of breath hydrogen and methane in healthy volunteers after ingestion of a commercial disaccharide mixture, Palatinit[®]. *Brit J Nutr* 54:389-400, 1985.
- Gibson GR, Cummings JH, Macfarlane GT, *et al*: Alternative pathways for hydrogen disposal during fermentation in the human colon. *Gut* 31:679-683, 1990.
- Gilat T, Ben Hur H, Gelman-Malachi E, *et al*: Alterations of the colonic flora and their effect on the hydrogen breath test. *Gut* 19:602-605, 1978.
- Grubler B, Ingwersen M, Meyer H, Schunemann C: *In vitro* investigations on gas formation from ileum and colon chyme in dogs. *Nutrition. Malnutrition and Dietetics in the Dog and Cat*. 34-36, 1990.
- Hammer HF: Colonic hydrogen absorption: quantification of its effect on hydrogen accumulation caused by bacterial fermentation of carbohydrates. *Gut* 34:818-822, 1993.
- Harries JT: Disorders of carbohydrate absorption. *Clinics in Gastroenterology* 11:17-30, 1982.
- Hawkins EC, Meric SM, Washabau RJ, *et al*: Digestion of bentriomide and absorption of xylose in healthy cats and absorption of xylose in cats with infiltrative intestinal disease. *Am J Vet Res* 47:567-569, 1986.
- Hertzler SR, Savaiano DA: Colonic adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance. *Am J Clin Nutr* 64:232-236, 1996.
- Hickey CA, Murphy EL, Calloway DH: Intestinal-gas production following ingestion of commercial wheat cereals and milling fractions. *Cereal Chemistry* 49:276-283, 1972.
- Hill FWG: Malabsorption syndrome in the dog: a study of thirty-eight cases. *J Sm Anim Pract* 13:575-594, 1972.
- Hirakawa M, Iida M, Kohroggi N, Fujishima M: Hydrogen breath test assessment of oro-caecal transit time: comparison with barium meal study. *Am J Gastroent* 83:1361-1363, 1988.
- Hoekstra JH, Vandenaker JHL: Facilitating effect of amino acids on fructose and sorbitol absorption in children. *J Pediatr Gastroent Nutr* 23:118-124, 1996.
- Holland RE, Herdt TH, Refsal KR: Breath hydrogen concentration and small intestinal malabsorption in calves. *Am J Vet Res* 47:2020-2024, 1986.

Holland RE, Herdt TH, Refsal KR: Pulmonary excretion of H₂ in calves with *Cryptosporidium*-induced malabsorption. *Dig Dis Sci* 34:1399-1404, 1989.

Jacobs RM, Norris AM, Lumsden JH, Valli VEO: Laboratory diagnosis of malassimilation. *Vet Clinics of North America: Small Animal Practice* 19:951-977, 1989.

Kerlin P, Wong L, Harris B, Capra S: Rice flour, breath hydrogen, and malabsorption. *Gastroenterology* 87:578-585, 1984.

Kerlin P, Wong L: Breath hydrogen testing in bacterial overgrowth of the small intestine. *Gastroenterology* 95:982-988, 1988.

King CE, Toskes PP: Small intestine bacterial overgrowth. *Gastroenterology* 76:1035-1055, 1979.

King CE, Toskes PP: Comparison of the 1-gram [¹⁴C]xylose, 10-gram lactulose-H₂, and 80-gram glucose-H₂ breath tests in patients with small intestine bacterial overgrowth. *Gastroenterology* 91:1447-1451, 1986.

Kotler DP, Holt PR, Rosensweig NS: Modification of the breath hydrogen test: increased sensitivity for the detection of carbohydrate malabsorption. *J Lab Clin Med* 100:798-805, 1982.

La Brooy SJ, Male PJ, Beavis AK, Misiewicz JJ: Assessment of the reproducibility of the lactulose H₂ breath test as a measure of mouth to caecum transit time. *Gut* 24:893-896, 1983.

Ladas SD, Giorgiotis K, Raptis SA: Breath hydrogen testing in small intestinal malabsorption. *Eur J Clin Invest* 19:A42, 1989.

Ladas SD, Giorgiotis K, Raptis SA: Complex carbohydrate malabsorption in exocrine pancreatic insufficiency. *Gut* 34:984-987, 1993.

Lajoie SF, Bank S, Miller TL, Wolin MJ: Acetate production from hydrogen and [¹³C] carbon dioxide by the microflora of human feces. *App Environ Microbiol* 54:2723-2727, 1988.

Levine AS, Levitt MD: Malabsorption of starch moiety of oats, corn and potatoes. *Gastroenterology* 80:1209, 1981.

Levitt MD, French P, Donaldson RM: Use of hydrogen and methane excretion in the study of the intestinal flora. *J Lab Clin Med* 72:988-989, 1968.

Levitt MD: Production and excretion of hydrogen gas in man. *New Eng J Med* 281:122-127, 1969.

Levitt MD, Donaldson RM: Use of respiratory hydrogen (H₂) excretion to detect carbohydrate malabsorption. *J Lab Clin Med* 75:937-945, 1970.

Levitt MD, Berggren T, Hastings J, Bond JH: Hydrogen (H₂) catabolism in the colon of the rat. *J Lab Clin Med* 84:163-167, 1974.

Levitt MD, Hirsh P, Fetzer CA, Sheahan M, Levine AS: H₂ excretion after ingestion of complex carbohydrates. *Gastroenterology* 92:383-389, 1987.

- Livesey G, Johnson IT, Gee JM, *et al*: Determination of sugar alcohol and polydextrose[®] absorption in humans by the breath hydrogen (H₂) technique: the stoichiometry of hydrogen production and the interaction between carbohydrates assessed *in vivo* and *in vitro*. *Eur J Clin Nutr* 47:419-430, 1993.
- Ludlow CL, Bruyette DS, Davenport DJ, Lieth DE: Daily and individual variation of breath hydrogen excretion in healthy dogs. *J Vet Int Med* 8:150, 1994a.
- Ludlow CL, Bruyette DS, Davenport DJ, Lieth DE: Relationship between breath hydrogen fraction and calculated breath hydrogen excretion in healthy dogs. *J Vet Int Med* 8:152, 1994b.
- Ludlow CL, Bruyette DS, Davenport DJ, Lieth DE: Dilution of mask collected breath hydrogen samples by dead space gases in healthy dogs. *J Vet Int Med* 9:191, 1995.
- McBurney MI, Thompson LU: *In vitro* fermentabilities of purified fiber supplements. *J Food Sci* 54:347-350, 1989.
- McBurney MI: Passage of starch into the colon of humans: quantification and implications. *Can J Physiol Pharmacol* 69:130-136, 1991.
- McIver MA, Redfield AC, Benedict EB: Gaseous exchange between the blood and the lumen of the stomach and intestines. *Am J Physiol* 76:92-111, 1926.
- Mackie RD, Levine AS, Levitt MD: Malabsorption of starch in pancreatic insufficiency. *Gastroenterology* 80:1220, 1981.
- MacLean WC, Fink BB: Lactose malabsorption by premature infants: magnitude and clinical significance. *J Pediatr* 97:383-388, 1980.
- Madsen JL, Larsen NE, Hilsted J, Worming H: Scintigraphic determination of gastrointestinal transit times: a comparison with breath hydrogen and radiologic methods. *Scand J Gastroenterol* 26:1263-1271, 1991.
- Mastropaolo G, Rees WDW: Evaluation of the hydrogen breath test in man: definition and elimination of the early hydrogen peak. *Gut* 28:721-725, 1987.
- Metz G, Jenkins DJA, Peters TJ, *et al*: Breath hydrogen as a diagnostic method for hypolactasia. *Lancet* 1:1155-1157, 1975.
- Metz G, Gassull MA, Leeds AR, *et al*: A simple method of measuring breath hydrogen in carbohydrate malabsorption by end-expiratory sampling. *Clinical Science Molecular Medicine* 50:237-240, 1976a.
- Metz G, Jenkins DJA, Newman A, Blendis LM: Breath hydrogen in hyposucrasia. *Lancet* 1:119-120, 1976b.
- Metz G, Gassull MA, Drasar BS, *et al*: Breath hydrogen test for small intestinal bacterial colonisation. *Lancet* 27:668-669, 1976c.
- Metz G, Jenkins JA, Blendis LM: Lactulose-hydrogen (H₂) breath test in health and disease. *Gut* 17:397-398, 1976d.
- Metz G, Jenkins DJA: Breath hydrogen during sleep. *Lancet* 1:145-146, 1977.

- Miller TL, Wolin MJ: Methanogens in human and animal intestinal tracts. *System Appl Microbiol* 7:223-229, 1986.
- Miller JB, Bokdam M, McVeagh P: Variability of breath hydrogen excretion in breast-fed infants during the first three months of life. *J Pediatr* 121:410-413, 1992.
- Mishkin S, Sablauskas L, Schwarcz J: "H₂ nonproducers" and malabsorption of carbohydrate. *Gastroenterology* 106:1726, 1993.
- Muir P, Papasouliotis K, Gruffydd-Jones TJ, *et al*: Evaluation of carbohydrate malassimilation and intestinal transit time in cats by measurement of breath hydrogen excretion. *Am J Vet Res* 52:1104-1109, 1991.
- Muir P, Gruffydd-Jones TJ, Cripps PJ, *et al*: Breath hydrogen excretion after oral administration of xylose to cats. *J Sm Anim Pract* 35:86-92, 1994.
- Muir P, Gruffydd-Jones TJ, Cripps PJ, Brown PJ: Breath hydrogen excretion by healthy cats after oral administration of oxytetracycline and metronidazole. *Vet Rec* 138:635-639, 1996.
- Murphy EL, Calloway DH: The effect of antibiotic drugs on the volume and composition of intestinal gas from beans. *Dig Dis* 17:639-642, 1972.
- Naylor JM, Liebel T: Effect of psyllium on plasma concentration of glucose, breath hydrogen, and faecal composition in calves with diarrhea treated orally with electrolyte solutions. *Am J Vet Res* 56:56-59, 1995.
- Newcomer AD, McGill DB, Thomas PJ, Hofmann AF: Prospective comparison of indirect methods for detecting lactase deficiency. *New Eng J Med* 293:1232-1235, 1975.
- Niu H, Schoeller DA, Klein PD: Improved gas chromatographic quantitation of breath hydrogen by normalization to respiratory carbon dioxide. *J Lab Clin Med* 94:755-763, 1979.
- Nordgaard I, Hansen BS, Mortensen PB: Colonic fermentation of complex dietary carbohydrates in short-bowel patients: no association with hydrogen excretion and fecal and plasma short-chain fatty acids. *Scand J Gastroenterol* 30:897-904, 1995.
- Olesen M, Rumessen JJ, Gudmand-Hoyer E: The hydrogen breath test in resistant starch research. *Eur J Clin Nutr* 46:S133-S134, 1992.
- Ostrander CR, Cohen RS, Hopper AO, *et al*: Breath hydrogen analysis: a review of the methodologies and clinical applications. *J Pediatr Gastroent Nutr* 2:525-533, 1983.
- Papasouliotis K, Muir P, Gruffydd-Jones TJ, *et al*: Decreased oro-caecal transit time, as measured by the exhalation of hydrogen, in hyperthyroid cats. *Res Vet Sci* 55:115-118, 1993a.
- Papasouliotis K, Muir P, Gruffydd-Jones TJ, *et al*: The effect of short-term dietary fibre administration on oro-caecal transit time in dogs. *Diabetologia* 36:207-211, 1993b.
- Papasouliotis K, Gruffydd-Jones TJ, Sparkes AH, Cripps P: Breath hydrogen (H₂) excretion after oral xylose to cats with chronic intestinal disease. *J Vet Int Med* 8:149, 1994.

- Papasouliotis K, Gruffyd-Jones TJ, Sparkes AH, Cripps PJ: A comparison of oro-caecal transit times assessed by the breath hydrogen test and the sulphasalazine/sulphapyridine method in healthy beagle dogs. *Res Vet Sci* 58:263-297, 1995.
- Payne DL, Welsh JD, Claypool PL: Breath hydrogen (H₂) response to carbohydrate malabsorption after exercise. *J Lab Clin Med* 102:147-150, 1983.
- Perman JA, Barr RG, Watkins JB: Sucrose malabsorption in children: noninvasive diagnosis by interval breath hydrogen determination. *J Pediatr* 93:17-22, 1978.
- Perman JA, Modler S, Olson AC: Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. *J Clin Invest* 67:643-650, 1981a.
- Perman JA, Waters LA, Harrison MR, *et al*: Breath hydrogen reflects canine intestinal ischemia. *Pediatr Res* 15:1229-1233, 1981b.
- Perman JA, Modler S: Glycoproteins as substrates for production of hydrogen and methane by colonic bacterial flora. *Gastroenterology* 83:388-393, 1982.
- Perman JA, Modler S, Barr RG, Rosenthal P: Fasting breath hydrogen concentration: normal values and clinical application. *Gastroenterology* 87:1358-1363, 1984.
- Perman JA, Modler S, Engel RR, Heldt G: Effect of ventilation on breath hydrogen measurements. *J Lab Clin Med* 105:436-439, 1985.
- Perman JA: Clinical applications of breath hydrogen measurements. *Can J Physiol Pharmacol* 69:111-115, 1991.
- Pitt P, de Bruijn KM, Beeching MF, *et al*: Studies on breath methane: the effect of ethnic origins and lactulose. *Gut* 21:951-959, 1980.
- Ravich WJ, Bayless TM, Thomas M: Fructose: incomplete intestinal absorption in humans. *Gastroenterology* 84:26-29, 1983.
- Read NW, Miles CA, Fisher D, *et al*: Transit of a meal through the stomach, small intestine, and colon in normal subjects and its role in the pathogenesis of diarrhea. *Gastroenterology* 79:1276-1282, 1980.
- Read NW, Al-Janabi MN, Bates TE, *et al*: Interpretation of the breath hydrogen profile obtained after ingesting a solid meal containing unabsorbable carbohydrate. *Gut* 26:834-842, 1985.
- Rhodes JM, Middleton P, Jewell DP: The lactulose hydrogen breath test as a diagnostic test for small bowel bacterial overgrowth. *Scand J Gastroenterol* 14:333-336, 1979.
- Richards EA, Stegгерdia FR: Production and inhibition of gas in various regions in the intestine of the dog. *Proc Soc Exp Biol Med* 122:573-576, 1966.
- Richards EA, Stegгерdia FR, Murata A: Relationship of bean substrates and certain intestinal bacteria to gas production in the dog. *Gastroenterology* 55:502-509, 1968.
- Riordan SM, McIver CJ, Walker BM, *et al*: The lactulose breath hydrogen test and small intestinal bacterial overgrowth. *Am J Gastroent* 91:1795-1803, 1996.

- Robb TA, Davidson GP: Advances in breath hydrogen quantitation in paediatrics: sample collection and normalization to constant oxygen and nitrogen levels. *Clin Chim Acta* 111:281-285, 1981.
- Rosado JL, Solomons NW: Storage of hydrogen breath-test samples in plastic syringes. *Clin Chem* 29:583-584, 1983.
- Ruge E: Beitrage zur kenntniss der damgase. *Chem Zentrabl* 7:347-351, 1862.
- Rumessen JJ, Kokholm G, Gudmand-Hoyer E: Methodological aspects of breath hydrogen (H₂) analysis. Evaluation of a H₂ monitor and interpretation of the breath H₂ test. *Scand J Clin Lab Invest* 47:555-560, 1987.
- Rumessen JJ, Hamberg O, Gudmand-Hoyer E: Influence of oro-caecal transit time on hydrogen excretion after carbohydrate malabsorption. *Gut* 30:811-814, 1989.
- Rumessen JJ, Hamberg O, Gudmand-Hoyer E: Interval sampling of end-expiratory hydrogen (H₂) concentrations to quantify carbohydrate malabsorption by means of lactulose standards. *Gut* 31:37-42, 1990.
- Rutgers HC, Batt RM, Elwood CM, Lamport A: Small intestinal bacterial overgrowth in dogs with chronic intestinal disease. *J Am Vet Med Assoc* 206:187-193, 1995.
- Rutgers HC, Batt RM, Sorensen SH, Fensome R: Breath hydrogen testing in the diagnosis and management of bacterial overgrowth in the dog. *BSAVA Congress*. 1996, pp 252.
- Saavedra JM, Perman JA: Current concepts in lactose malabsorption and intolerance. *Annu Rev Nutr* 9:475-502, 1989.
- Saltzberg DM, Levine GM, Lubar C: Impact of age, sex, race, and functional complaints on hydrogen (H₂) production. *Dig Dis Sci* 33:308-313, 1988.
- Sarno S, Erasmus LP, Haslbeck M, Holzl R: Orocaecal transit-time by the H₂ method: effects of definitions of caecal entry and test meal. *Ital J Gastroenterol* 25:55-64, 1993.
- Schlesinger DP, Rubin SI, Papich MG, Hamilton DL: Use of breath hydrogen measurement to evaluate oro-caecal transit time in cats before and after treatment for hyperthyroidism. *Can J Vet Res* 57:89-94, 1993.
- Shermak MA, Saavedra JM, Jackson TL, *et al*: Effect of yogurt on symptoms and kinetics of hydrogen production in lactose-malabsorbing children. *Am J Clin Nutr* 62:1003-1006, 1995.
- Simpson KW, Batt RM, Jones D, *et al*: Effects of exocrine pancreatic insufficiency and replacement therapy on the bacterial flora of the duodenum in the dog. *Am J Vet Res* 51:203-206, 1990.
- Simpson KW: Small intestinal bacterial overgrowth. *J Am Vet Med Assoc* 205:405-407, 1994.
- Solomons NW, Viteri F: Breath hydrogen during sleep. *Lancet* 2:636, 1976.
- Solomons NW, Viteri FE, Hamilton LH: Application of a simple gas chromatographic technique for measuring breath hydrogen. *J Lab Clin Med* 90:856-862, 1977.

- Solomons NW, Viteri F: Development of an interval sampling hydrogen (H₂) breath test for carbohydrate malabsorption in children: evidence for a circadian pattern of breath H₂ concentration. *Pediat Res* 12:816-823, 1978.
- Solomons NW, Garcia R, Schneider R, *et al*: H₂ breath tests during diarrhea. *Acta Paediatr Scand* 68:171-172, 1979.
- Sparkes AH, Pappasoulotis K, Viner J, *et al*: Assessment of oro-caecal transit time in cats by the breath hydrogen method: the effects of sedation and a comparison of definitions. *Res Vet Sci* 60:243-246, 1996.
- Spohr A, Guilford WG, Haslett S, Vibe-Peterson G: Use of breath hydrogen to screen for disaccharidase deficiency in dogs. Unpublished data, 1996.
- Staniforth DH, Rose D: Statistical analysis of the lactulose/breath hydrogen test in the measurement of oro-caecal transit: its variability and predictive value in assessing drug action. *Gut* 30:171-175, 1989.
- Stevenson DK, Cohen RS, Ostrander CR, *et al*: A sensitive analytical apparatus for measuring hydrogen production rates. II. Application to studies in human infants. *J Pediatr Gastroenterol Nutr* 1:233-237, 1982.
- Strocchi A, Ellis C, Levitt MD: Reproducibility of measurements of trace gas concentrations in expired air. *Gastroenterology* 101:175-179, 1991.
- Strocchi A, Levitt MD: Factors affecting hydrogen production and consumption by human fecal flora: the critical roles of hydrogen tension and methanogenesis. *J Clin Invest* 89:1304-1311, 1992a.
- Strocchi A, Levitt MD: Maintaining intestinal H₂ balance: credit the colonic bacteria. *Gastroenterology* 102:1424-1426, 1992b.
- Strocchi A, Corazza G, Ellis CJ, *et al*: Detection of malabsorption of low doses of carbohydrate: accuracy of various breath H₂ criteria. *Gastroenterology* 105:1404-1410, 1993a.
- Strocchi A, Ellis CJ, Levitt MD: Use of metabolic inhibitors to study H₂ consumption by human faeces: evidence for a pathway other than methanogenesis and sulfate reduction. *J Lab Clin Med* 121:320-327, 1993b.
- Thompson DG, Binfield P, De Belder A, *et al*: Extraintestinal influences on exhaled breath hydrogen measurements during the investigation of gastrointestinal disease. *Gut* 26:1349-1352, 1985.
- Thompson DG, O'Brien JD, Hardie JM: Influence of the oropharyngeal microflora on the measurement of exhaled breath hydrogen. *Gastroenterology* 91:853-860, 1986.
- Thompson DG: The clinical application of exhaled breath hydrogen testing for the study of gastrointestinal motility, in Read NW (ed): *Gastrointestinal Motility: Which Test?* Wrightson Biomedical Publishers Ltd, 1989, pp 143-147.
- Ushijima K, Riby JE, Kretchmer N: Carbohydrate malabsorption. *Pediatrics Clinics North America* 42:899-912, 1995.

Vogelsang H, Ferenci P, Frotz S, *et al*: Acidic colonic microclimate-possible reason for false negative hydrogen breath tests. *Gut* 29:21-26, 1988.

Washabau RJ, Strombeck DR, Buffington CA, Harrold D: Evaluation of intestinal carbohydrate malabsorption in the dog by pulmonary hydrogen gas excretion. *Am J Vet Res* 47:1402-1406, 1986a.

Washabau RJ, Strombeck DR, Buffington CA, Harrold D: Use of pulmonary hydrogen gas excretion to detect carbohydrate malabsorption in dogs. *J Am Vet Med Assoc* 189:674-679, 1986b.

Williams DA, Batt RM, McLean LEFT: Bacterial overgrowth in the duodenum of dogs with exocrine pancreatic insufficiency. *J Am Vet Med Assoc* 191:201-205, 1987.

Williams DA, Guilford WG: Procedures for the evaluation of pancreatic and gastrointestinal tract diseases, in Guilford WG, Center SA, Strombeck DR, Williams DA, Meyer DJ (eds): *Strombeck's Small Animal Gastroenterology, 3rd Ed.* W.B. Saunders, 1996. pp 77-113.

Zentek J, Meyer H, Schwartz-Hafter G: Localization of H₂-generation and -absorption in the alimentary tract of dogs. *J Vet Nutr* 2:11-15, 1993.

CHAPTER 3

EFFECT OF FOOD PARTICLE SIZE ON CARBOHYDRATE ASSIMILATION ASSESSED BY BREATH HYDROGEN TESTING IN DOGS

Introduction

Carbohydrate malabsorption occurs in many gastrointestinal diseases due to an interruption in the normal degradative and absorptive processes of this nutrient. Impaired intraluminal digestion due to pancreatic amylase deficiency is commonly seen in dogs with exocrine pancreatic insufficiency (Hill 1972, Washabau *et al* 1986b). In addition, impaired brush border digestion of ingested oligosaccharides or starch breakdown products, and impaired monosaccharides absorption may occur with a variety of small intestinal disorders (Batt and Hall 1989, Ushijima *et al* 1995, Williams 1996). Diets containing highly digestible carbohydrate sources are commonly fed to dogs with these diseases to minimise the amount of malabsorbed carbohydrate and associated clinical signs of carbohydrate intolerance.

Starch is the principal carbohydrate source in canine diets, therefore the digestibility of starch is a major determinant of the amount of carbohydrate that will be malabsorbed. Starch digestibility is influenced by a variety of factors (Dreher *et al* 1984, Rooney and Pflugfelder 1986, Biliaderis 1991, Cummings and Englyst 1995). These include intrinsic factors which relate to the composition and physical form of the starch fed, and extrinsic factors which primarily relate to the gastrointestinal handling of the food. Important intrinsic factors affecting starch digestibility include the botanical source of the starch, the effects of food processing and food storage (food particle size, degree of starch disruption, gelatinisation and retrogradation), and the presence of lipid/protein interactions, nonstarch polysaccharides and α -amylase inhibitors which may retard starch digestion. Examples of extrinsic factors that may affect the degree of starch digestion include the extent of chewing, the orocolic transit time, the intestinal concentrations of digestive enzymes, and the amount of starch and other food components consumed.

Differences in susceptibility to enzymatic attack between different botanical sources of starch primarily relates to differences in the structure of the starch granule. Native starch granules are highly organised structures containing randomly orientated semi-crystalline regions

in an amorphous matrix. The crystalline regions are formed by amylose and amylopectin chains organised in left-handed, parallel-stranded double helices, connected and stabilised by hydrogen bonds. In the amorphous areas intermolecular hydrogen bonding is reduced with less association between the macromolecular chains (Guilbot and Mercier 1985). Digestion of the starch granule generally begins with amylase attack at the starch granule surface, and proceeds preferentially in the amorphous areas since the crystalline regions are the most resistant to amylolysis (Franco *et al* 1992, Gallant *et al* 1992). Factors implicated in the susceptibility of native starch granules to enzymatic hydrolysis include: size of the starch granule (Franco *et al* 1992), porosity of the starch granule (Colonna *et al* 1992), amylose content (Behall *et al* 1989, Cone and Wolters 1990), degree and type of crystallinity (Ring *et al* 1988), and morphology and ultrastructure of the starch granule (Gallant *et al* 1992).

Food processing techniques generally alter the structure of native starch granules to favour more rapid amylolysis. The application of water and heat to starch causes starch granule swelling, breakage of the intermolecular hydrogen bonds, dissociation of the macromolecular chains, and loss of the crystalline structure of the granule. This process is referred to as gelatinisation (Guilbot and Mercier 1985, Annison and Topping 1994). Gelatinisation has a profound affect on the digestibility of starch, resulting in a greatly increased susceptibility to enzymatic degradation. Apart from gelatinisation, food processing techniques may also increase the digestibility of starch. Food processing may disrupt physical barriers (eg., fibre) that render starch inaccessible to enzymatic hydrolysis (Snow and O'Dea 1981, Wursh *et al* 1986, Bjork *et al* 1994, Livesey *et al* 1995), induce physical injury to the starch granules referred to as "damaged starch" (Evers and Stevens 1985, Stark and Yin 1986), and reduce the size (and therefore increase enzyme accessibility) of food particles (O'Dea *et al* 1981, Snow and O'Dea 1981, Heaton *et al* 1988, Schweizer *et al* 1988).

Breath H₂ studies have been widely applied to the investigation of carbohydrate malabsorption in humans (Perman 1991, Strocchi and Levitt 1991) and more recently in animals (Washabau *et al* 1986a, Holland *et al* 1986, Muir *et al* 1991, Bracher *et al* 1995). H₂ exhaled in the breath is primarily derived from the microbial fermentation of carbohydrates in the colon, and may therefore be used to evaluate the amount of carbohydrate escaping digestion and absorption in the small bowel (Levitt and Donaldson 1970). It has been shown that there is a linear correlation between the quantity of carbohydrate malabsorbed and the amount of H₂ excreted in the breath (Bond and Levitt 1972, Fritz *et al* 1985, Washabau *et al* 1986a, Rumessen *et al* 1990). The objective of this study was to utilise measurements of exhaled breath H₂ to determine if the particle size of a carbohydrate source influences carbohydrate assimilation in healthy dogs.

Materials and Methods

Animals

Seventeen healthy male and female Border collie cross dogs were used for the study. These dogs were aged from 2 to 6 years, and weighed between 16 and 34 kg. The dogs were supplied by the Animal Health Services Centre of Massey University, and were cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals. The dogs were considered healthy on the basis of a normal physical examination and lack of history and clinical signs of gastrointestinal disease. The experimental protocol was approved by the Massey University Animal Ethics Committee.

Study protocol

The two carbohydrate sources used in this study were white rice^a and corn^b. These carbohydrates were chosen as they are highly digestible carbohydrates commonly used in veterinary therapeutic diets for gastrointestinal disease. The rice was cooked in boiling water for twelve minutes and fed whole or blended. The blended rice was prepared by blending cooked rice in a food processor for one minute, until the rice was of paste-like consistency. The corn was cooked in boiling water for three minutes and fed chopped or blended. The corn kernels were individually chopped into halves or blended as described for the rice. The corn kernels were halved rather than fed whole to reduce the possibility of the fibrous hulls interfering with digestive enzyme access to the corn starch.

The dogs were fasted for 24 hours prior to feeding to reduce the effect of past meals on baseline breath H₂ results. The two rice meals were fed to ten dogs in a randomised cross over fashion, 24 hours apart. The same procedure was repeated for the two corn meals eight weeks later (three of the ten dogs used in the rice study were also used in the corn study). The carbohydrate content of the test meal was fed at half daily maintenance energy requirements calculated by the formula $\text{kcal} = (\text{kg}^{0.75}) \times 66$. Thirty grams of bacon fat^c was added to the test meals (in addition to the 50% kcal carbohydrate component) to increase palatability. Dogs that did not willingly consume the test meals within 10 minutes were force fed. This was only necessary in 2 cases, and in both dogs the whole and blended carbohydrate diets were force fed.

^a Budget Long Grain Rice: Safeway Traders Ltd, Wellington, New Zealand

^b Choice Golden Kernels Sweet Corn: Watties Frozen Foods Ltd, Auckland, New Zealand

^c A. E. Preston & Co Ltd, New Zealand

Breath collection and analysis

Breath samples were collected prior to feeding (baseline), every 90 minutes for 18 hours, and at 24 hours after each test meal. Mixed expired breath was collected via an anaesthetic face mask technique originally described for use in children (Solomons *et al* 1977), and previously applied successfully to dogs, cats and calves (Washabau *et al* 1986a, Holland *et al* 1986, Muir *et al* 1991). A close fitting anaesthetic face mask was placed over the muzzle and attached to a 4 litre capacity anaesthetic reservoir bag via a non-rebreathing valve^d. Dead space in the collection apparatus was minimised by allowing the dogs to breathe through the system, during which time the rebreathing bag was removed, emptied and reattached while the dog continued to breathe through the face mask and non-rebreathing valve. After refilling the anaesthetic reservoir bag, an expired air sample was aspirated directly from the base of the bag into a 20 ml syringe via a three-way stop cock attached firmly at the base of the bag. The plastic syringe was sealed with a needle embedded in a rubber stopper so that it was air tight. The basic equipment used to collect breath samples and its application to dogs, is pictured in Figure 3.1.

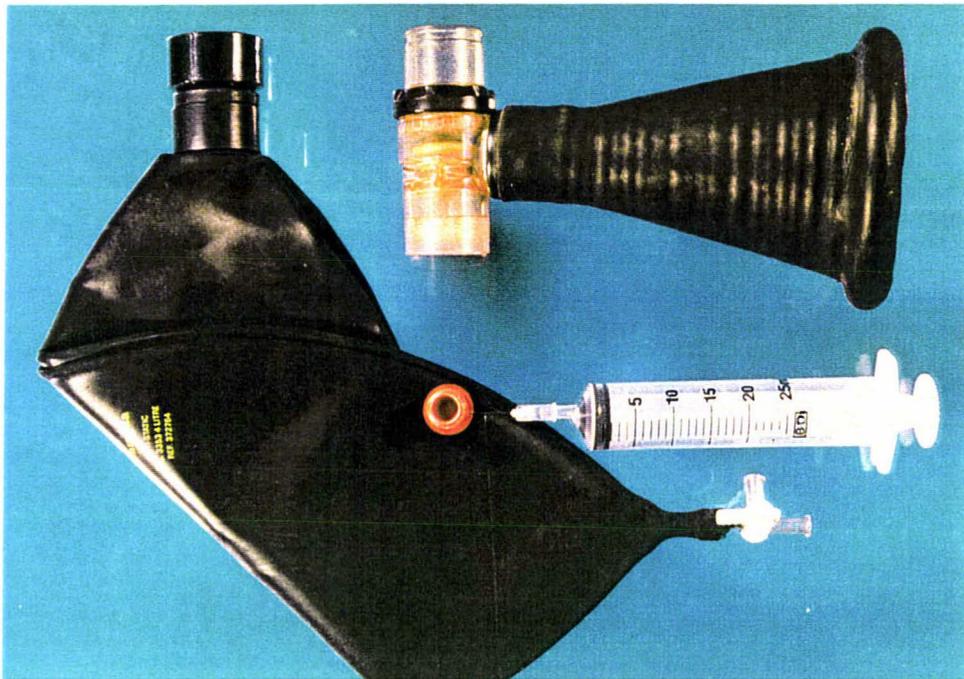
The breath collection procedure was repeated at each time point so that duplicate samples were obtained. Duplicate samples are necessary to detect sampling errors (eg., gas leakage from syringe, flatulence contamination, large ventilation changes, poor collection procedure) and to minimise the effect of large moment to moment variations in breath H₂. Dilution of H₂ in expired air was further minimised by housing the dogs in small runs during the test period to restrict exercise, and by handling the dogs carefully to minimise excitement during breath collections. Both these practices were introduced to reduce hyperventilation which may markedly decrease breath H₂ concentrations (Payne *et al* 1983, Perman *et al* 1985). A summary of the breath collection protocol used in this study is presented in Table 3.1.

Breath samples were analysed within twelve hours of collection, over which period the loss of H₂ gas has been shown to be minimal (Ellis *et al* 1988,^e). An electrochemical cell calibrated with a standard gas mixture was used for the analysis of H₂ concentration (ppm) in the breath samples. The accuracy of this meter is ± 1 ppm for the range of H₂ concentrations measured in this study.

^d E-valve: Ambu International, Copenhagen, Denmark

^e Guilford WG: unpublished observations, 1992

^f GMI Exhaled Hydrogen Monitor: GMI Medical Ltd, Renfrew, Scotland



A



B

Figure 3.1 The collection of expired breath by the face mask technique. (A) Basic equipment required for expired breath collection including: an anaesthetic face mask; a non-rebreathing valve; an anaesthetic reservoir bag; a three way stop-cock; and a plastic syringe sealed with a rubber stopper. (B) The breath collection apparatus applied to a dog

Table 3.1 Protocol used to obtain expired breath samples by the anaesthetic face mask technique

1. Avoid any exercise or excitement of dogs presampling
2. If increased activity/excitement occurs then calm the dog for 3-5 minutes prior to beginning sampling
3. Fit face mask with attached rebreathing valve and reservoir bag firmly to the dog's muzzle
4. Fill the reservoir bag, detach bag, empty bag, and refill bag
5. Remove the entire apparatus from the dog's face and withdraw a sample from the three way stop-cock at the base of the bag into a plastic syringe
6. Seal syringe firmly, immediately after collecting sample
7. Repeat step 3-6

Note: If obvious changes in ventilation rates occur during sampling (ie., deep breath that fills a large volume of the bag) detach bag, empty, reattach & begin collection again.

Light microscopy

Light microscopy was used to assess the degree of starch granule disruption in the carbohydrate meals after blending. Impression smears were made of the whole and blended rice and the chopped and blended corn. All the samples were stained with 1% potassium iodine and 1% potassium iodide[§]. The average number of intact starch granules per high power field were determined from ten separate fields of the smears, and the amount of exuded starch present (starch that had leached out of the granule form) was assessed.

Statistical analysis

The mean total area under the breath H₂ curves (AUC_{tot}) and the mean area under the breath H₂ curve up to six hours after feeding (AUC_{6h}) were determined for each test meal. The mean of the maximum breath H₂ concentrations of each dog (peak breath H₂ concentration) and the mean time at which these maximum breath H₂ values occurred (time of peak breath H₂ concentrations) were also determined for each test meal. In addition, the maximum point of the mean breath H₂ curve and the time at which it occurred was determined for each test meal. The AUC_{tot} and the AUC_{6h} of the blended rice were compared with the AUC_{tot} and AUC_{6h} of the whole rice, as was the peak breath H₂ concentration and time of peak breath H₂ concentrations. The same comparisons were made for the corn meals. All comparisons were made using the Wilcoxon signed-rank test. A non-parametric method of statistical analysis was chosen because of the small sample size.

[§] Lugol solution[®]: Sigma, St Louis, Missouri

Results

Rice

The mean \pm SD areas under the curves, peak breath H₂ concentrations, and times of the peak breath H₂ concentrations for both the whole and blended rice are displayed in Table 3.2. The total area under the breath H₂ curve (AUC_{tot}), and the peak breath H₂ concentration following the whole rice, were found to be significantly greater ($p < 0.01$ & $p < 0.006$ respectively) than that of the blended rice. The area under the breath H₂ curve over the first 6 hours following feeding (AUC_{6h}), and the time of the peak breath H₂ concentrations were not significantly different ($p > 0.2$ & $p > 0.1$ respectively) between the two rice meals.

Table 3.2 Mean \pm SD breath H₂ data of 10 dogs after the ingestion of a whole rice or blended rice meal

	<i>AUC_{6h}</i> (<i>ppm.h</i>)	<i>AUC_{tot}</i> (<i>ppm.h</i>)	<i>Peak breath H₂</i> (<i>ppm</i>) [#]	<i>Time of peak</i> <i>breath H₂</i> (<i>hours</i>)
Whole Rice	15.52 \pm 15.73	111.86 \pm 81.92*	11.80 \pm 7.40**	10.50 \pm 3.08
Blended Rice	9.04 \pm 6.88	39.71 \pm 20.74*	4.40 \pm 2.09**	8.55 \pm 2.14

* significantly different $p < 0.01$; ** significantly different $p < 0.006$; # represents the mean of the 10 dogs' individual peak breath H₂ concentrations (in contrast to the maximum point of the mean breath H₂ curve in Fig. 3.1)

The mean \pm SEM breath H₂ concentrations of ten dogs over 24 hours after the ingestion of whole and blended cooked rice are presented in Figure 3.2. The maximum point of the mean breath H₂ curve \pm SEM was 10.20 \pm 2.60 ppm at 9 hours after the whole rice meal, and 3.80 \pm 0.73 ppm at 7.5 hours after the blended rice meal.

Light microscopy of the whole and blended rice revealed a larger average number of intact starch granules per high power field (hpf) of the whole rice (78 intact starch granules/hpf) in comparison to the blended rice (32 intact starch granules/hpf). More of the starch present in the blended rice smear was in the form of exuded starch (Fig 3.3).

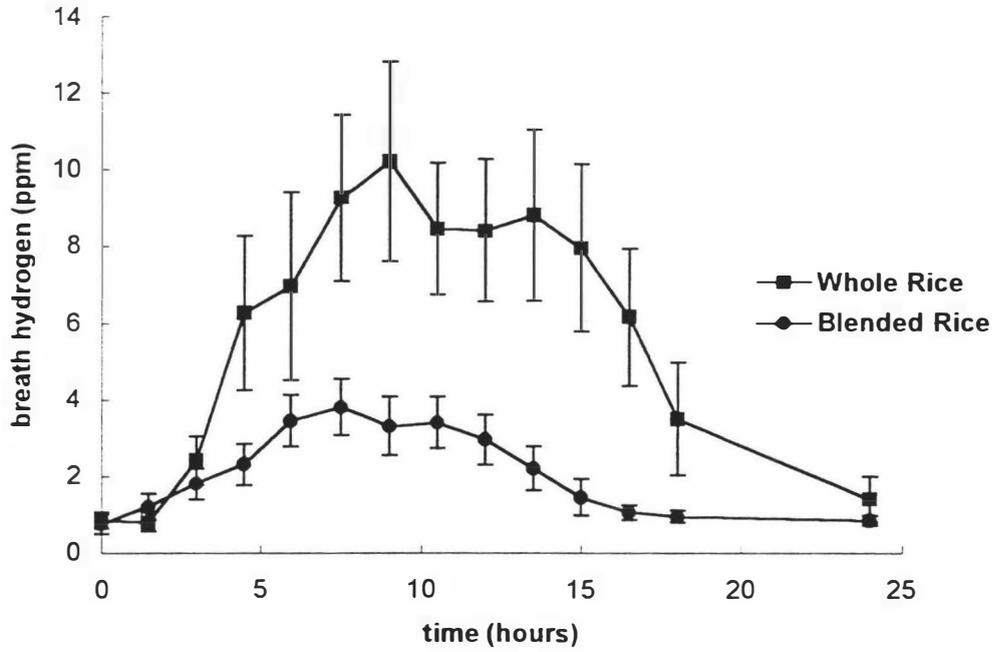


Figure 3.2 Mean \pm SEM breath H₂ concentrations in 10 dogs after the ingestion of a whole rice or blended rice meal

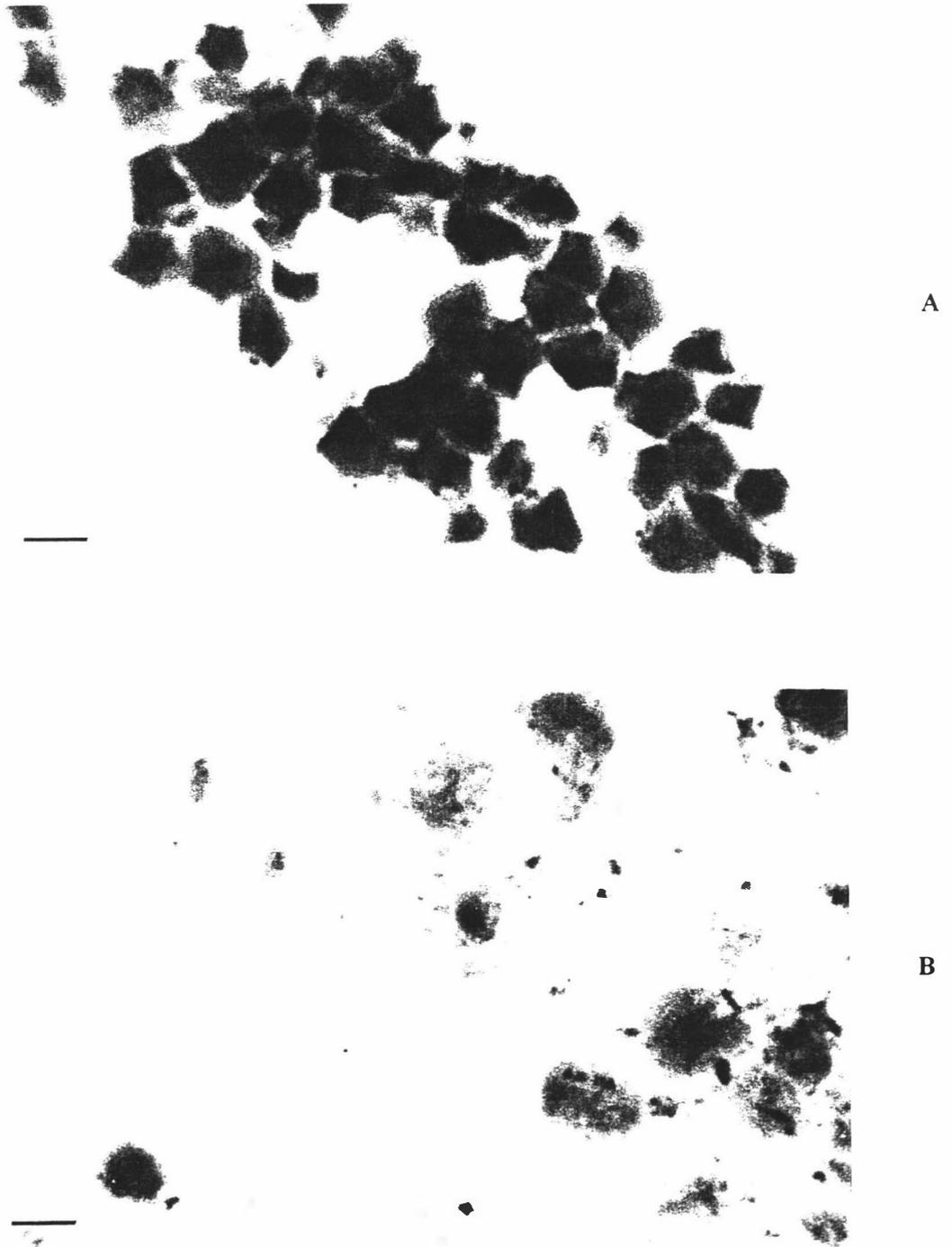


Figure 3.3 Photomicrographs of cooked rice starch from two different rice meals (Bar present = $5\mu\text{m}$). (A) rice starch from a whole rice grain is largely in the form of intact starch granules. (B) rice starch from blended rice has mostly exuded and dispersed from the starch granule form

Corn

The mean \pm SD areas under the curves, peak breath H₂ concentrations, and times of peak breath H₂ concentrations for both the chopped and blended corn meals are displayed in Table 3.3. In contrast to the results for the rice, the total area under the breath H₂ curve (AUC_{tot}) and the peak breath H₂ concentration were not significantly different ($p > 0.1$ & $p > 0.5$ respectively) between the chopped and blended corn meals. However, the area under the breath H₂ curve over the first 6 hours following the blended corn meal (AUC_{6h}) was significantly greater ($p < 0.02$) than that of the chopped corn meal. In addition, the time of the peak breath H₂ concentrations of the blended corn was significantly shorter ($p < 0.05$) than that of the chopped corn.

Table 3.3 Mean \pm SD breath H₂ data of 10 dogs after the ingestion of a chopped corn or blended corn meal

	<i>AUC_{6h}</i> (ppm.h)	<i>AUC_{tot}</i> (ppm.h)	<i>Peak breath</i> H ₂ (ppm) [#]	<i>Time of peak</i> breath H ₂ (hours)
Chopped Corn	7.69 \pm 4.05*	97.12 \pm 78.25	11.70 \pm 10.10	10.65 \pm 2.49**
Blended Corn	19.91 \pm 12.58*	64.84 \pm 42.06	9.85 \pm 4.16	6.90 \pm 2.93**

*significantly different $p < 0.02$; ** significantly different $p < 0.05$; # represents the mean of the 10 dogs' individual peak breath H₂ concentrations (in contrast to the maximum point of the mean breath H₂ curve in Fig. 3.3)

The mean \pm SEM breath H₂ concentrations of ten dogs over 24 hours after the ingestion of chopped and blended cooked corn are shown in Figure 3.4. The maximum point of the mean breath H₂ curve \pm SEM was 13.00 \pm 4.30 ppm at 9 hours after the chopped corn meal, and 8.5 \pm 1.62 ppm at 7.5 hours after the blended corn meal.

Light microscopy of the corn starch revealed similar amounts of both intact starch granules and exuded starch in the chopped corn (19 intact starch granules/hpf) and blended corn (17 intact starch granules/hpf) smears (Fig 3.5).

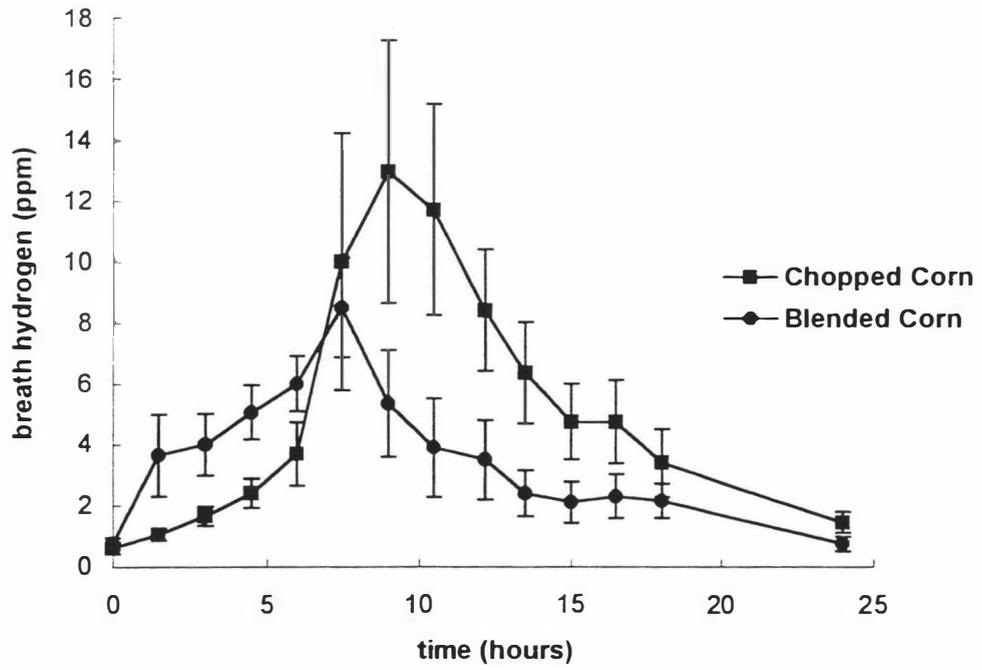


Figure 3.4 Mean \pm SEM breath H₂ concentrations of 10 dogs after the ingestion of a chopped corn or blended corn meal

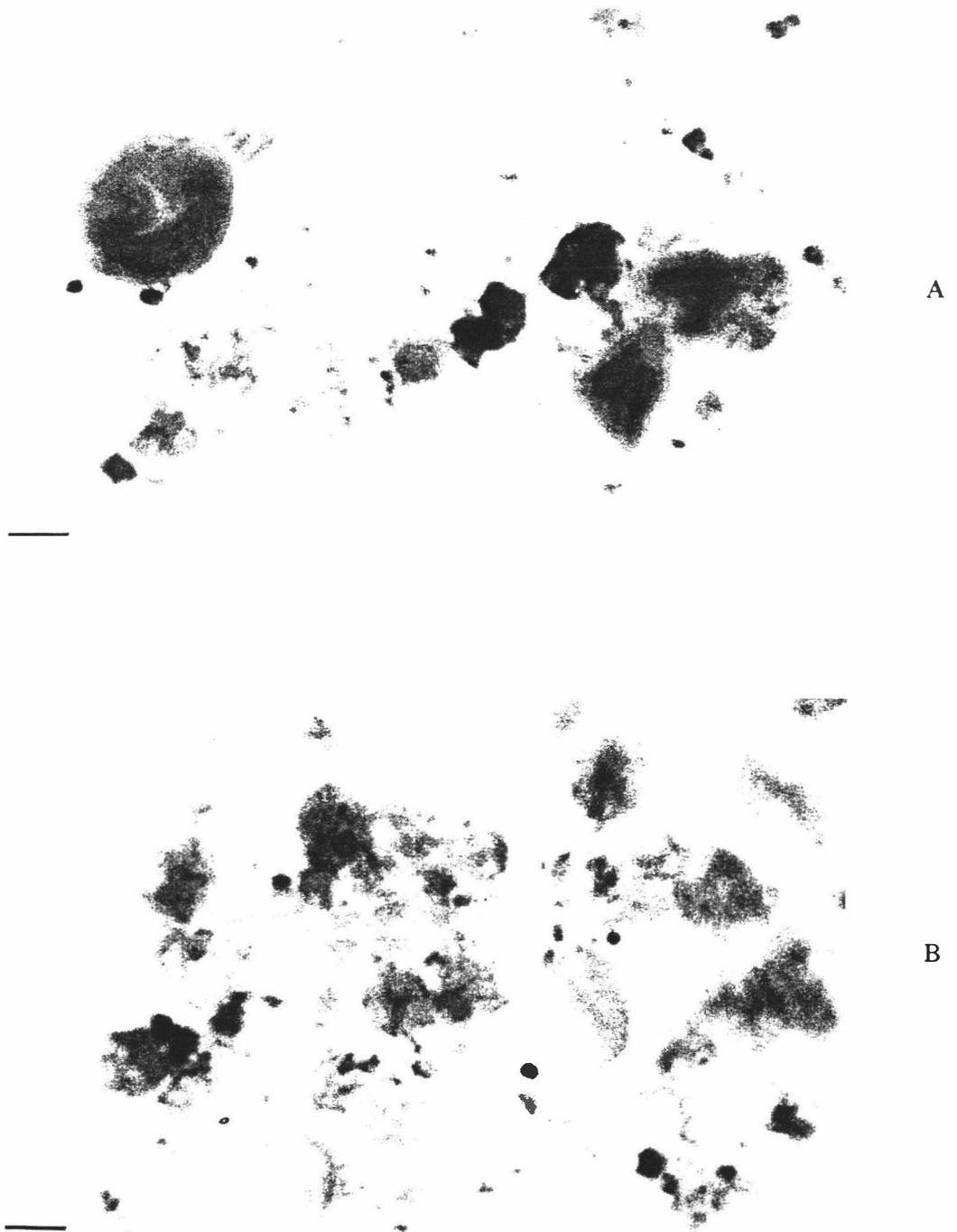


Figure 3.5 Photomicrographs of cooked corn starch from two different corn meals (Bar present = $5\mu\text{m}$). (A) corn starch from a chopped corn kernel and (B) corn starch from blended corn kernels. Both micrographs contain similar amounts of intact starch granules and exuded/dispersed starch

Discussion

The increased AUC_{tot} and peak breath H_2 concentration for the whole rice in comparison to the blended rice meal suggests that the blended rice has been more completely assimilated in the small intestine, with less carbohydrate reaching the colon for fermentation. Alternatively, it is possible that the difference in particle size could have resulted in different degrees of colonic fermentation. This latter possibility seems unlikely, however, as smaller particles would be expected to be fermented to a greater extent than larger particles (Macfarlane and Cummings 1991), and is in contrast to the results in the present study where the blended rice produced less H_2 in the breath than the whole rice. The more efficient assimilation of the blended rice meal could be attributed to reduced food particle size per se and/or structural starch granule damage created during the blending process. The decreased number of intact starch granules and increased amount of exuded starch on light microscopy of the blended rice in comparison to the whole rice suggests that significant disruption of the starch granules was present which probably aided digestion.

It is often difficult to separate the effects on carbohydrate assimilation of decreasing food particle size and the disruption of food structure because the processing used to cause one usually results in the other. Smaller food particles are thought to be more rapidly and effectively hydrolysed than are larger particles due to the increased surface area available for enzymatic attack (O'Dea *et al* 1981, Snow and O'Dea 1981, Heaton *et al* 1988). This theory certainly seems to be true for native starch granules, as smaller granules have been shown to be more extensively hydrolysed than larger ones from the same source (Franco *et al* 1992). In support of this theory, it has also been shown that an increased rate of starch hydrolysis in people occurs with smaller grain size and finer flours (Heaton *et al* 1988), dry-milled vs rolled raw cereals and blended vs unblended moist starchy foods (Schweizer *et al* 1988), cereal flours vs rolled cereals (Snow and O'Dea 1981), and ground vs whole rice (O'Dea *et al* 1981). Levitt *et al* (1987) also demonstrated increased digestive efficiency of smaller carbohydrate particles (refined flours vs whole oats and wheat) using breath H_2 testing in man. In most of these studies, however, disruption of fibre and damaged starch may have added significantly to the increased rate of digestion.

The results for the chopped and blended corn meals were in contrast to the results for the rice meals. The lack of difference between the two corn meals in AUC_{tot} and peak breath H_2 concentration suggests that the degree of assimilation of both corn meals was similar, and therefore the effect of particle size and food structure disruption was not so important for the

digestion of corn in comparison to that of rice. Alternatively, the blended corn reaching the colon may have been fermented to a greater extent than the chopped corn resulting in overall similar breath H_2 levels in spite of differences in assimilation.

Reduction of food particle size and mechanical disruption of food does not always result in more efficient digestion, as has been demonstrated with oats and lentils in man (Heaton *et al* 1988, Jenkins *et al* 1982). It is likely that when food is already highly digestible (or extremely indigestible), the effects of particle size and mechanical disruption will be less apparent. Light microscopy of the corn starch from both chopped and blended corn meals appeared similar, with both samples containing moderate amounts of intact starch granules and exuded starch. This suggests that prior to blending, the cooking processes (and possibly the halving of the corn kernels) caused significant starch disruption. Although the breath H_2 results of the chopped and blended corn meals in this study suggest similar degrees of starch assimilation, it is still possible that the blended corn may have been assimilated to a slightly greater extent. More complete fermentation of the blended corn may have masked differences in assimilation between the corn groups. Also, the number of dogs used in the study was small, and a conservative statistical analysis was used. Alternative tests such as *in vitro* rates of digestion or blood glucose and insulin curves may have been useful to assess if a difference in the rapidity of digestion was present between the chopped and blended corn (Casiraghi *et al* 1992, Livesey *et al* 1995).

The greater AUC_{6h} and earlier time of the peak breath H_2 concentrations seen with the blended corn, suggests that blended corn has a shorter orocolic transit time, and/or faster fermentability than chopped corn. A faster orocolic transit time of the blended corn would be feasible given the more liquid appearance of the blended corn meal. While blending rice resulted in a thick and sticky paste, the blended corn assumed a more liquid form, and it is well recognised that liquids pass through the gastrointestinal tract more rapidly than solids (Kerlin and Phillips 1983, Van den Brom and Happe 1986, Hornof *et al* 1989). Alternatively, the structure of the chopped corn kernels may have delayed bacterial degradation of the chopped corn, thereby slowing fermentation and contributing to the later breath H_2 rise seen with the chopped corn in comparison to the blended corn.

In summary, the effects of decreasing the food particle size of rice and corn fed to healthy dogs appears to influence the degree of assimilation of rice, and the gastrointestinal transit time and/or fermentability of corn. These findings are likely to be similar or more pronounced in dogs with carbohydrate malabsorption and as such have implications for the formulation of therapeutic diets.

References

- Annisson G, Topping DL: Nutritional role of resistant starch: chemical structure vs physiological function. *Annu Rev Nutr* 14:297-320, 1994.
- Batt RM, Hall EJ: Chronic enteropathies in the dog. *J Small Anim Pract* 30:3-12, 1989.
- Behall KM, Scholfield DJ, Yuhaniak I, Canary J: Diets containing high amylose vs amylopectin starch: effects on metabolic variables in human subjects. *Am J Clin Nutr* 49:337-344, 1989.
- Biliaderis CG: The structure and interactions of starch with food constituents. *Can J Physiol Pharmacol* 69:60-78, 1991.
- Bjorck I, Granfeldt Y, Liljeberg H, et al: Food processing affecting the digestion and absorption of carbohydrates. *Am J Clin Nutr* 59:699S-705S, 1994.
- Bond JH, Levitt MD: Use of pulmonary hydrogen (H₂) measurements to quantitate carbohydrate absorption: study of partially gastrectomized patients. *J Clin Invest* 51:1219-1225, 1972.
- Bracher V, Steiger R, Huser S: Preliminary results of a combined xylose absorption/hydrogen exhalation test in horses. *Schweiz Arch Tierh* 137:297-305, 1995.
- Casiraghi MC, Brighenti F, Pellegrini N, et al: Effect of processing on rice starch digestibility evaluated by *in vivo* and *in vitro* methods. *J Cereal Sci* 17:147-156, 1992.
- Colonna P, Leloup V, Buleon A: Limiting factors of starch hydrolysis. *Eur J Clin Nutr* 46:S17-S32, 1992.
- Cone JW, Wolters MGE: Some properties and degradability of isolated starch granules. *Starch* 42:298-301, 1990.
- Cummings JH, Englyst HN: Gastrointestinal effects of food carbohydrate. *Am J Clin Nutr* 61:938S-945S, 1995.
- Dreher ML, Dreher CJ, Berry JW: Starch digestibility of foods: a nutritional perspective. *Crit Rev Food Sci Nutr* 20:47-71, 1984.
- Ellis CJ, Kneip JM, Levitt MD: Storage of breath samples for H₂ analyses. *Gastroenterology* 94:822-824, 1988.
- Evers AD, Stevens DJ: Starch Damage. In: Pomeranz Y (ed) *Adv Cereal Sci Technol, Vol. VII*. 1985, pp 321-349.
- Franco CML, do Rio Preto SJ, Ciacco CF, Geraldo-Campinas B: Factors that affect the enzymatic degradation of natural starch granules- effect of size of the granules. *Starch* 44:422-426, 1992.
- Fritz M, Siebert G, Kasper H: Dose dependence of breath hydrogen and methane in healthy volunteers after ingestion of a commercial disaccharide mixture, Palatinit[®]. *Brit J Nutr* 54:389-400, 1985.

- Gallant DJ, Bouchet B, Buleon A, Perez S. Physical characteristics of starch granules and susceptibility to enzymatic degradation. *Eur J Clin Nutr* 46:S3-S16, 1992.
- Guilbot A, Mercier C: Starch. In: Aspinall GO (ed) *The Polysaccharides, Vol 3*. Academic Press, Orlando, 1985. pp 210-282.
- Hill FWG: Malabsorption syndrome in the dog: a study of thirty-eight cases. *J Small Anim Pract* 13:575-594, 1972.
- Heaton KW, Marcus SN, Emmett PM, Bolton CH: Particle size of wheat, maize, and oat test meals: effects on plasma glucose and insulin response and on rate of starch digestion *in vitro*. *Am J Clin Nutr* 47:675-682, 1988.
- Holland RE, Herdt TH, Refsal KR: Breath hydrogen concentration and small intestinal malabsorption in calves. *Am J Vet Res* 47:2020-2024, 1986.
- Hornof WJ, Kablik PD, Strombeck DR *et al*: Scintigraphic evaluation of solid-phase gastric emptying in the dog. *Vet Radiol* 30:242-248, 1989.
- Jenkins DJA, Thorne MJ, Camelon K, *et al*: Effect of food processing on digestibility and blood glucose response: a study of lentils. *Am J Clin Nutr* 36:1093-1101, 1982.
- Kerlin P, Phillips S: Differential transit of liquids and solid residue through the human ileum. *Am J Physiol* 245:G38-G43, 1983.
- Levitt MD, Donaldson RM. Use of respiratory hydrogen (H₂) excretion to detect carbohydrate malabsorption. *J Lab Clin Med* 75:937-945, 1970.
- Levitt MD, Hirsh P, Fetzer CA, *et al*: H₂ excretion after ingestion of complex carbohydrates. *Gastroenterology* 92:383-389, 1987.
- Livesey G, Wilkinson JA, Roe M, Faulks R, *et al*: Influence of the physical form of barely grain on the digestion of its starch in the human small intestine and implications for health. *Am J Clin Nutr* 61:75-81, 1995.
- Macfarlane GT, Cummings JH: The colonic flora, fermentation, and large bowel digestive function. In: Phillips SF, Pemberton JH, Shorter RG (eds) *The Large Intestine: Physiology Pathophysiology, and Disease*. Raven Press Ltd, New York, 1991. pp 51-92.
- Muir P, Papassouliotis K, Gruffydd-Jones TJ, *et al*: Evaluation of carbohydrate malassimilation and intestinal transit time in cats by measurement of breath hydrogen excretion. *Am J Vet Res* 52:1104-1109, 1991.
- O'Dea K, Snow P, Nestel P: Rate of starch hydrolysis *in vitro* as a predictor of metabolic responses to complex carbohydrate *in vivo*. *Am J Clin Nutr* 34:1991-1993, 1981.
- Perman JA: Clinical application of breath hydrogen measurements. *Can J Physiol Pharmacol* 69:111-115, 1991.
- Ring SG, Gee JM, Whittam M, *et al*: Resistant starch: its chemical form in foodstuffs and effect on digestibility *in vitro*. *Food Chem* 28:97-109, 1988.

- Rooney LW, Pflugfelder RL: Factors affecting starch digestibility with special emphasis on sorghum and corn. *J Anim Sci* 63:1607-1623, 1986.
- Schweizer TF, Reimann S, Wursch P: Definition and measurement of a starch digestion index and a study of factors determining starch digestion rates in foods. *Lebensm Wiss u Technol* 21:352-357, 1988.
- Snow P, O'Dea K: Factors affecting the rate of hydrolysis of starch in food. *Am J Clin Nutr* 34:2721-2727, 1981.
- Solomons NW, Viteri FE, Hamilton LH: Application of a simple gas chromatographic technique for measuring breath hydrogen. *J Lab Clin Med* 90:856-862, 1977.
- Solomons NW, Viteri F, Rosenberg IH: Development of an interval sampling hydrogen (H₂) breath test for carbohydrate malabsorption in children: evidence for a circadian pattern of breath H₂ concentration. *Pediat Res* 12:816-823, 1978.
- Stark JR, Yin XS: The effect of physical damage on large and small barely starch granules. *Starch* 38:369-374, 1986.
- Strocchi A, Levitt MD: Measurement of starch absorption in humans. *Can J Physiol Pharmacol* 69:108-110, 1991.
- Ushijima K, Riby JE, Kretchmer N: Carbohydrate malabsorption. *Pediat Clin N Am* 4:899-913, 1995.
- Washabau RJ, Strombeck DR, Buffington CA, Harrold DS: Evaluation of intestinal carbohydrate malabsorption in the dog by pulmonary hydrogen gas excretion. *Am J Vet Res* 47:1402-1406, 1986a.
- Washabau RJ, Strombeck DR, Buffington CA, Harrold DS: Use of pulmonary hydrogen gas excretion to detect carbohydrate malabsorption in dogs. *J Am Vet Med Assoc* 6:674-679, 1986b.
- Williams DA: Malabsorption, small intestinal bacterial overgrowth, and protein-losing enteropathy. In: Guilford WG, Center SA, Strombeck DR, Williams DA, Meyer DJ (eds) *Strombeck's Small Animal Gastroenterology, 3rd ed.* WB Saunders, Philadelphia, 1996. pp 367-380.
- Wursh P, Del Vedovo S, Koellreutter B: Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *Am J Clin Nutr* 43:25-29, 1986.

CHAPTER 4

EFFECT OF 5% DEHYDRATION ON BREATH HYDROGEN CONCENTRATIONS IN DOGS

Introduction

Breath H₂ testing is becoming a clinically useful method of detecting carbohydrate malabsorption, small intestinal bacterial overgrowth (SIBO), and abnormal orocolic transit times (OCTT) in animals (Washabau *et al* 1986, Holland *et al* 1986, Muir *et al* 1994, Rutgers *et al* 1995, Schlesinger *et al* 1993, Papasouliotis *et al* 1995). Unfortunately, numerous variables may influence the results of breath H₂ tests. The majority of these variables have been identified in humans, however their occurrence is likely to be similar in animals. The variables can be broadly classified into gastrointestinal and extraintestinal factors. Gastrointestinal factors include: numbers and metabolic activities of microbes present in the gastrointestinal tract that produce and consume H₂ (Strocchi and Levitt 1992a &b), presence of oropharangeal fermentation and/or gastro-ileal reflex of fermentable substrates into the colon (Thompson *et al* 1985 & 1986, Read *et al* 1985), rate of colonic entry of fermentable substrates (Read *et al* 1985), colonic pH (Perman *et al* 1981, Florent *et al* 1985, Vogelsang *et al* 1988) adequacy of colonic perfusion (Perman *et al* 1981), and presence of diarrhea (Solomons *et al* 1979). Extraintestinal factors which may influence breath H₂ readings include: amount and type of substrate/food consumed (Bond and Levitt 1972, Washabau *et al* 1986, Christl *et al* 1992, Olesen *et al* 1992), technique of expired air collection (Strocchi *et al* 1991, Ludlow *et al* 1995), ventilation rates (Metz and Jenkins 1977, Payne *et al* 1983, Perman *et al* 1985), antibiotic therapy (Gilat *et al* 1978, Richards and Steggerdia 1966, Muir *et al* 1996), enema procedures prior to breath testing (Gilat *et al* 1978), anxiety (Calloway and Murphy 1968), and age of the subject undergoing testing (Stevenson *et al* 1982, Saltzberg *et al* 1988).

Although the list of variables that are known to influence breath H₂ test results are extensive, one variable of unknown importance is that of hydration status. Many of the dogs admitted to veterinary hospitals for breath H₂ testing are likely to be suboptimally hydrated due to the loss of fluid and electrolytes that occurs with anorexia, vomiting, and/or diarrhea. The purpose of the present study was to determine if mild dehydration (5%) influences breath H₂ measurements in dogs.

Materials and Methods

Animals

Ten healthy Border collie-cross dogs (nine female, one male) were used for the study. These dogs were aged from 7 months to 10 years, and weighed between 12 and 25 kg. The dogs were supplied by the Animal Health Services Centre of Massey University, and were cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals. The dogs were considered to be healthy on the basis of a normal physical examination and lack of clinical signs of gastrointestinal disease. The experimental protocol was approved by the Massey University Animal Ethics Committee.

Study protocol

Breath H₂ tests were performed on dogs when they were fully hydrated and on the same dogs when they were 5% dehydrated. The study was performed in a randomised, balanced, cross-over fashion, in a two stage process (see statistical design & analysis). All dogs received a three day rest period (during which time they received their usual daily diet) between finishing one test period and beginning food withdrawal for the next test period.

Five percent dehydration was defined as a loss of 5% of the dog's normal body weight following food and water deprivation. "Normal" body weights were obtained after a 12 hour overnight fast and outdoor walk to encourage urination and defecation. Body weights were determined to the nearest 0.05 kg using electronic weighing scales, and were monitored every 4-6 hours (after the first 12 hours) during the water deprivation period. Test meals were fed to the water deprived dogs when they had lost 5% of their normal body weight (38-56 hours after water removal, or 50-68 hours after food withdrawal). Laboratory parameters taken to monitor the hydration status of food and water deprived dogs included: blood urea nitrogen concentrations (BUN), packed cell volumes (PCV), serum total protein concentrations (TP), and urine specific gravities (USG). These parameters were determined at baseline, when the dogs had lost 2.5-3.0% of their bodyweight, and midway (9 hours) through the breath H₂ testing period.

The fully hydrated dogs had food withheld for a similar period (52-68 hours) to the dehydrating dogs before feeding the test meal, but water was available *ad libitum*. Healthy dogs have been reported to lose approximately 0.5-1.75% body weight per day during the first 4-9 days of starvation (Prentiss *et al* 1959, Brady *et al* 1977, de Brunijine and Lubberink 1977). Based on these findings, we allowed the non-dehydrated dogs to lose up to 1.5% body weight

per day (maximum cumulative loss of 3%) during the fasting period before supplementing these dogs with water via gavage (enough water was given to reduce daily loss of bodyweight to 1.5%). This was done to ensure the dogs remained well hydrated during the fasting period, and because pilot studies had revealed that some dogs with water freely available chose not to drink even when their body weights fell by as much as 4% in 24 hours. In the non-dehydrated dogs, body weights were monitored every 12 hours, and BUN, PCV, TP and USG were determined at baseline and midway through (9 hours) the breath H₂ test period.

The test meal used in the study consisted of 25% maintenance energy requirements (MER) of a commercial dog food^a mixed with 25% MER of kibbled wheat.^b Therefore, the total meal accounted for 50% of the dogs' daily MER, which was calculated by the formula $(\text{kg}^{0.75}) \times 66$. Kibbled wheat was added to the dog roll to increase H₂ production and excretion in the breath, as pilot studies had demonstrated that dogs fed the dog roll exclusively produced only minimal breath H₂ levels.

Breath collection and analysis

Expired breath samples were collected prior to feeding (baseline), and every 90 minutes for 18 hours after the test meal. See Chapter 3 for details regarding the collection and analysis of breath samples.

Statistical design

A two stage, balanced, cross-over, repeated measures design was employed, where six dogs were tested in the first stage, and conditional on the results for the first stage, four more dogs were added. Two stage designs for general linear models are outlined in principle, for the case where each stage has the same sample size, in Spurrier (1982). Two stage designs are characterised by taking a small initial sample size in the first stage and allowing one of three possible decisions at the end of this phase: accept the null hypothesis of no treatment difference, proceed to the second stage, or reject the null hypothesis. The boundaries for the decision in this case were set at: $p > 0.3$ accept the null hypothesis, $0.3 \geq p \geq 0.01$ proceed to stage two, and $p < 0.01$ reject the null hypothesis, where p is the estimated probability of no treatment difference. Two stage designs have a lower expected sample size, which can be a considerable advantage in animal studies where costs are high and/or ethical considerations important.

^a Champ chunky chicken and vegetable dog roll, Best Friend Pet Foods, New Zealand

^b Kibbled wheat, Woolworths Ltd, New Zealand

Statistical analysis

Duplicate breath H₂ samples at each time point were averaged, except for five time points in the dehydrated group of the dogs in stage one. These five duplicate readings were extremely high readings, and were considered to be outliers due to contamination of the breath samples by flatulence. Expired breath samples were considered to be contaminated by flatus when duplicate samples differed from each other by >50% and when readings were >100 ppm. In previous experiments and pilot studies, we have found that duplicates normally vary by <30% and expired air normally contains <50 ppm with this test meal. The outliers were excluded from the raw data, and at these time points values were either determined from the duplicate sample (if uncontaminated) or, if both duplicates were considered outliers, a value was calculated from the average of the preceding and following time points. This was only necessary for 5 of 130 time points, and alternative adjustments had little effect on significance levels. Furthermore, the adjustment procedure was conservative, in that it reduced the difference seen between the dehydrated and non-dehydrated dogs' breath H₂ readings. Outliers due to flatulence obtained in stage two of the study were replaced with repeat samples that were collected within 5 minutes of the contaminated reading.

The area under the breath H₂ curve over the 18 hour test period (AUC) was determined for each dog in each treatment group. Analysis of the AUC figures at the end of stage one indicated that the difference between AUCs for the dehydrated and non-dehydrated groups fell into the intermediate range ($p = 0.081$), and the experiment proceeded to stage two where four more dogs were added to the study. For two stage designs, the significance level cannot be estimated using standard statistical software. The distribution of the usual test of treatment differences is not an F statistic, since the test at the second stage only proceeds if the (F) test at the first stage falls in the intermediate range. Significance levels were thus determined by simulation via Proc IML, the interactive matrix procedure of SAS^c using the estimated variances for dog, dog x treatment, and error, under the assumption of no treatment differences. The experiment was simulated 10,000 times using these estimated values, and the probability of the null hypothesis being true estimated from the percentiles for the test statistic from the simulations.

$$\text{ie., } P_c = P(F_1 < C_1) + P(C_1 < F_1 < C_2 \text{ and } F_{12} < C_3)$$

where; P denotes probability,

P_c = conditional probability null hypothesis is accepted given null hypothesis true,

F_1 = test statistic at end of stage one

^c SAS/IML software, SAS Institute, Cary, NC, USA.

C_1 = 70th percentile of the relevant F distribution under the null hypothesis at the end of stage one,

C_2 = 99th percentile for the same F distribution as for C_1 ,

C_3 = actual value of the test statistic F_{12} from the experiment at the end of stage two.

The second term on the right hand side of the equation for P_c (ie., $F_{12} < C_3$) is determined from the simulations. Note that P_c can also be written:

$$P_c = P(F_1 < C_1) + P(C_1 < F_1 < C_2) P(F_{12} < C_3 | C_1 < F_1 < C_2)$$

where: $P(F_{12} < C_3 | C_1 < F_1 < C_2)$ = probability that $F_{12} < C_3$ given F_1 is in the range $C_1 < F_1 < C_2$.

$$\begin{aligned} \text{ie., } P_c &= 0.7 + (0.99 - 0.7) P(F_{12} < C_3 | C_1 < F_1 < C_2) \\ &= 0.7 + 0.29 P(F_{12} < C_3 | C_1 < F_1 < C_2). \end{aligned}$$

Note also that $1 - P_c$ is the probability of rejecting the null hypothesis given that it is true.

An ad hoc test was used to test whether there was a significant difference in the number of times breath samples were considered to be falsely elevated by flatus between the dehydrated and non-dehydrated dogs. A one sided sign test was used. A two stage test was not conducted because the statistical power of the test was too small given the number of flatulence readings at the end of stage one.

Except for stage one of the experiment, all comparisons were considered significant at $p < 0.050$.

Results

The clinical and laboratory parameters (used to monitor hydration status) of the ten dogs collected midway through the breath H_2 testing period (47-65 hours after water withdrawal) are presented in Table 4.1. During the water deprivation period, all the dogs lost $\geq 5\%$ of their body weight within 38-56 hours of water removal (ie, 0 hour of the breath H_2 testing period), and did not loose more than 7% of their body weight by the end of the 18 hour breath H_2 test period.

The mean \pm SEM breath H_2 concentrations of the ten dogs when fully hydrated and when 5% dehydrated after the ingestion of the test meal are presented in Figure 4.1. The mean \pm SEM breath H_2 concentrations peaked at 34.9 ± 3.2 ppm at 10.5 hours, and 29.3 ± 2.6 ppm at 12 hours for the dehydrated and non-dehydrated dogs respectively.

The mean \pm SD area under the breath H_2 concentration vs time curve (AUC) over 18 hours following the ingestion of the test meal was found to be 251.2 ± 73.2 ppm.h for the non-dehydrated dogs, and 359.1 ± 117.8 ppm.h for the same dogs tested when they were 5%

dehydrated. The AUC for the dehydrated dogs was found to be significantly greater ($p = 0.0173$) than the AUC when the dogs were not dehydrated. Figure 4.2 displays the breath H_2 AUC data for the ten dogs tested under normal and dehydrated conditions. It can be seen that all dogs except one (dog 6) exhaled more breath H_2 following the test meal when dehydrated.

Table 4.1. Clinical and laboratory parameters of dehydrated and non-dehydrated dogs

Dog No.	Weight loss (%) [#]		BUN (mmol/l)		PCV (%)		TP (g/dl)		USG	
	ND	D	ND	D	ND	D	ND	D	ND	D
1	3.0*	5.6	5.5	7.2	48	43	6.2	7.0	1.024	1.040
2	3.0*	5.3	4.31	5.8	44	46	6.0	7.2	1.027	1.042
3	3.0*	5.8	5.56	5.93	44	44	5.8	6.8	1.030	1.049
4	3.0*	5.2	4.61	6.32	43	43	6.0	6.0	1.025	1.065
5	3.0*	5.3	5.17	6.68	44	48	6.2	6.2	1.023	1.060
6	3.0*	5.0	3.33	3.33	43	48	6.4	6.8	1.036	1.057
7	2.9	5.5	5.12	5.81	38	44	6.2	7.8	1.031	1.036
8	3.0*	5.0	6.25	7.4	43	49	6.8	7.2	1.034	1.058
9	3.0*	5.6	6.02	9.11	46	47	6.5	7.1	1.037	1.055
10	2.6	5.0	5.72	5.18	48	52	6.6	7.1	**	**

D dehydrated; ND non-dehydrated; [#] 50-68 hours after food withdrawal (0 hour of breath H_2 test period); * dogs supplemented with water; ** measurements not taken.

Contamination of breath samples by flatulence was detected in six of the ten dogs tested. In the majority of samples considered to be contaminated by flatulence, the breath H_2 concentration was greater than 200 ppm, and in some samples exceeded 1000 ppm. Of the six dogs with breath samples contaminated by flatulence, five of these dogs had contaminated samples during the dehydration test period only. In the remaining dog, flatulence was detected during both test periods. The number of breath samples contaminated by flatulence was found to be significantly greater ($p = 0.0312$) when the dogs were 5% dehydrated, compared to when they were fully hydrated.

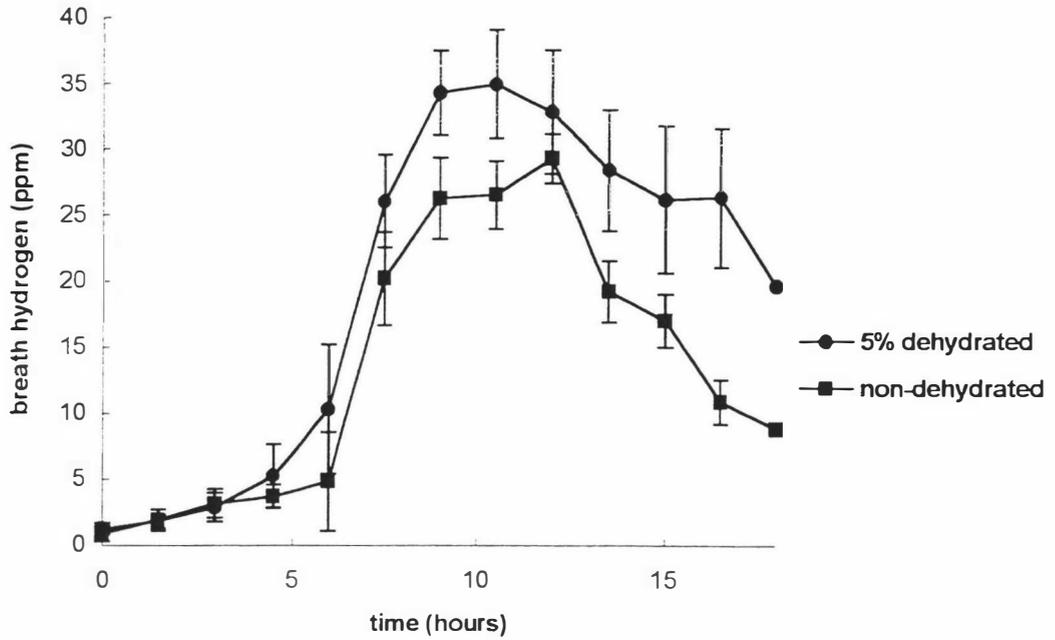


Figure 4.1 Mean \pm SEM breath H₂ concentrations of 10 dehydrated and non-dehydrated dogs following the ingestion of a wheat-containing test meal

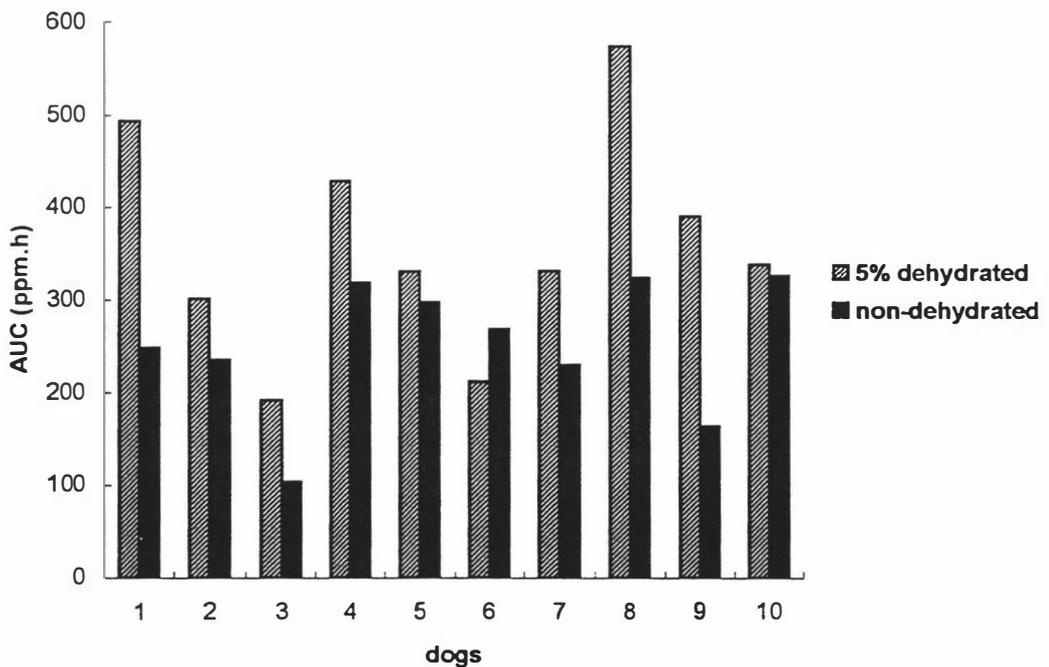


Figure 4.2 Area under the breath H₂ curve (AUC) in 10 dogs tested when dehydrated and non-dehydrated over 18 hours following the ingestion of a wheat-containing test meal

Discussion

The average daily weight loss of approximately 2-3 % observed in the dogs in the present study is consistent with that reported in other studies in which healthy dogs have been deprived of water (Prentiss *et al* 1959, Hardy and Osborne 1979). Body weight loss of greater than 3% during the first few days of starvation despite free access to water has been observed by Brady *et al* (1977), and was also observed in this study, particularly in lean dogs. The changes in laboratory parameters of the dogs during the dehydration and non-dehydration test periods reflected the difference in hydration between the groups at the time of breath H₂ testing. Mild increases in in BUN, PCV, and serum TP over baseline (but never outside the reference range) were identified in the majority of the dehydrated dogs. Urine specific gravity measures increased to ≥ 1.040 in all of the dogs in which USG was measured, except in one dog (dog 7). This dog was 10 years of age, and may have had subclinical renal disease. These USG changes are similar to reports from previous studies (Hardy and Osborne 1979, Balazs *et al* 1971). Interestingly, we found that dogs either tended to concentrate their urine very effectively (eg., >1.050) with little changes in PCV or serum TP, or had less concentrated urine but much greater rises in serum TP, and occasionally PCV. This probably relates to individual differences in renal function, renal responsiveness to antidiuretic hormone (ADH), or secretion of ADH.

Significantly increased concentrations of H₂ were found in the expired breath of dogs when they were 5% dehydrated in comparison to when they were fully hydrated. This increase in breath H₂ concentrations may be attributed to either a total increase in “net” H₂ production within the gastrointestinal tract, or to proportionally more H₂ being exhaled in the breath (despite similar levels of intestinal H₂ production) through changes in the rate of gas diffusion to mucosal blood. Increased production of intestinal H₂ could be a consequence of greater substrate availability (eg., more carbohydrate malabsorbed), or may occur due to changes in intestinal microbial numbers and/or metabolic activities. Water is an essential component of fermentation systems (Hobson and Wheatley 1993), and it is likely that dehydrating the dogs to 5%, resulted in a drier bowel lumen. Bacterial species that produce H₂ may have preferred these drier conditions, or alternatively, luminal water availability may have been sufficient for H₂ production but insufficient for further fermentation reactions necessary for H₂ consumption.

The rate of gas diffusion from the gastrointestinal tract is determined by a complex interplay of factors including: partial pressure differences, diffusion distances (membrane thickness and surface area), gas diffusivity, gas solubility in tissues and blood, mucosal capillary blood flow, and countercurrent exchange efficiency along the intestinal villus (Levitt *et*

al 1981). It is possible that increased H₂ diffusion from the gastrointestinal lumen under dehydrated conditions could occur as a result of reduced thickness of the unstirred water layer, given that aqueous regions usually present the most significant diffusion barrier to gases of high lipid solubility such as H₂ (Dietschy *et al* 1971, Levitt *et al* 1981). Mucosal damage may also promote gas diffusion, but this was unlikely to be present at the degree of dehydration induced in this study. Furthermore, mucosal damage is most likely to occur due to impaired mucosal blood flow, however, this has been associated with decreased rather than increased breath H₂ excretion in cats (McIver *et al* 1926) and dogs (Perman *et al* 1981).

Ventilation changes may also influence the amount of H₂ excreted in the breath. Hypoventilation, which reduces the dilution of H₂ in the alveoli, has been associated with elevated breath H₂ measurements in people (Metz and Jenkins 1977, Perman *et al* 1985). This may have occurred in the dehydrated dogs due to inactivity. However, the dogs were kept in small cages, and activity levels were minimal during both test periods. Furthermore, hyperventilation is probably a more likely scenario than hypoventilation in dehydrated animals due to the development of metabolic acidosis.

Despite the possibility that increased H₂ diffusion to mucosal blood may account for the greater breath H₂ concentrations found in the dehydrated dogs, the concurrent finding of greater flatulence detection in this group supports the alternative theory of an increase in intestinal H₂ production. The presence of high numbers of flatus contaminated breath samples was an incidental finding in this study, but had been observed occasionally during other tests.^d In humans, flatus passage is associated with peak breath H₂ excretion (Calloway and Murphy 1968) and a linear correlation between the volume of H₂ excreted in the breath and flatus exists (Hammer 1993). As intestinal H₂ production increases, the percentage of intestinal H₂ exhaled in the breath decreases in comparison to that excreted rectally (Christl *et al* 1992, Hammer 1993). Therefore, the presence of significantly greater amounts of flatus detected during the dehydration test period suggests the presence of larger amounts of intestinal H₂, rather than simply an increase in the amount of H₂ diffusing out of the gastrointestinal lumen into mucosal blood.

In conclusion, this study demonstrates that mild dehydration results in significantly increased breath H₂ concentrations. This is clinically relevant because many animals undergo breath H₂ tests to investigate gastrointestinal complaints that can result in suboptimal hydration. The results of this study suggest it is advisable to correct a patient's hydration deficit prior to breath H₂ testing to maximise the specificity of the test.

^d Bissett SA: personal observations, 1996.

References

- Balazs T, Sekella R, Pauls JF: Renal concentration test in beagle dogs. *Lab Anim Sci* 21:546-548, 1971.
- Bond JH, Levitt MD: Use of pulmonary hydrogen measurements to quantitate carbohydrate absorption. *J Clin Invest* 51:1219-1225, 1972.
- Brady LJ, Armstrong MK, Muiruri KL, *et al*: Influence of prolonged fasting in the dog on glucose turnover and blood metabolites. *J Nutr* 107:1053-1060, 1977.
- Calloway DH, Murphy EL: The use of expired air to measure intestinal gas formation. *Ann New York Acad Sc* 150:82-95, 1968.
- Christl SU, Murgatroyd PR, Gibson GR, Cummings JH: Production, metabolism, and excretion of hydrogen in the large intestine. *Gastroenterology* 102:1269-1277, 1992.
- de Brunijne JJ, Lubberink AME: Obesity. In: Kirk RW (ed) *Current Veterinary Therapy VI*. WB Saunders Company, Philadelphia, 1977. pp 1068-1070.
- Dietschy JM, Sallee VL, Wilson FA: Unstirred water layers and absorption across the intestinal mucosa. *Gastroenterology* 61:932-934, 1971.
- Florent C, Flourie B, Leblond A, *et al*: Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an *in vivo* study). *J Clin Invest* 75:608-613, 1985.
- Gilat T, Ben Hur H, Gelman-Malachi E, *et al*: Alterations of the colonic flora and their effect on the hydrogen breath test. *Gut* 19:602-605, 1978.
- Hammer HF: Colonic hydrogen absorption: quantification of its effect on hydrogen accumulation caused by bacterial fermentation of carbohydrates. *Gut* 4:818-822, 1993.
- Hardy RM, Osborne CA: Water deprivation test in the dog: maximal normal values. *J Am Vet Med Assoc* 174:479-483, 1979.
- Hobson PN, Wheatley AD, (eds): *Anaerobic Digestion: Modern Theory and Practice*. Elsevier Science Publishers Ltd, London, 1993. pp 7-72.
- Holland RE, Herdt TH, Refsal KR: Breath hydrogen concentration and small intestinal malabsorption in calves. *Am J Vet Res* 47:2020-2024, 1986.
- Levitt MD, Bond JH, Levitt DG: Gastrointestinal gas. In: Johnson LR (ed) *Physiology of the Gastrointestinal Tract*, Raven Press, New York, 1981. pp 1301-1316.
- Ludlow CL, Bruyette DS, Davenport DJ, Lieth DE: Dilution of mask collected breath hydrogen samples by dead space gases in healthy dogs. *J Vet Int Med (abstract)* 9:191, 1995.
- McIver MA, Redfield AC, Benedict EB: Gaseous exchange between the blood and the lumen of the stomach and intestines. *Am J Physiol* 76:92-111, 1926.
- Metz G, Jenkins DJA: Breath hydrogen during sleep. *Lancet* 1:145-146, 1977.

- Muir P, Gruffydd-Jones TJ, Cripps PJ, *et al*: Breath hydrogen excretion after oral administration of xylose to cats. *J Sm Anim Pract* 35:86-92, 1994.
- Muir P, Gruffydd-Jones TJ, Cripps PJ, Brown PJ: Breath hydrogen excretion by healthy cats after oral administration of oxytetracycline and metronidazole. *Vet Rec* 138:635-639, 1996.
- Olesen M, Rumessen JJ, Gudmand-Hoyer E: The hydrogen breath test in resistant starch research. *Eur J Clin Nutr* 46:S133-S134, 1992.
- Papasouliotis K, Gruffydd-Jones TJ, Sparkes AH, Cripps PJ: A comparison of oro-caecal transit times assessed by the breath hydrogen test and the sulphasalazine/sulphapyridine method in healthy beagle dogs. *Res Vet Sci* 58:263-297, 1995.
- Payne DL, Welsh JD, Claypool PL: Breath hydrogen (H₂) response to carbohydrate malabsorption after exercise. *J Lab Clin Med* 102:147-150, 1983.
- Perman JA, Modler S, Olson AC: Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. *J Clin Invest* 67:643-650, 1981.
- Perman JA, Waters LA, Harrison MR, *et al*: Breath hydrogen reflects canine intestinal ischemia. *Pediatr Res* 15:1229-1233, 1981.
- Perman JA, Modler S, Engel RR, Heldt G: Effect of ventilation on breath hydrogen measurements. *J Lab Clin Med* 105:436-439, 1985.
- Prentiss PG, Wolf AV, Eddy HA: Hydroponia in cat and dog. Ability of the cat to meet its water requirements solely from a diet of fish or meat. *Am J Physiol* 196:625-632, 1959.
- Read NW, Al-Janabi MN, Bates TE, *et al*: Interpretation of the breath hydrogen profile obtained after ingesting a solid meal containing unabsorbable carbohydrate. *Gut* 26:834-842, 1985.
- Richards EA, Steggerdia FR: Production and inhibition of gas in various regions in the intestine of the dog. *Proc Soc Exp Biol Med* 122:573-576, 1966.
- Rutgers HC, Batt RM, Entwistle M, *et al*: Breath hydrogen testing in the diagnosis of bacterial overgrowth in the dog. *J Vet Int Med (abstract)* 9:191, 1995.
- Saltzberg DM, Levine GM, Lubar C: Impact of age, sex, race, and functional complaints on hydrogen (H₂) production. *Dig Dis Sci* 33:308-313, 1988.
- Schlesinger DP, Rubin SI, Papich MG, Hamilton DL: Use of breath hydrogen measurement to evaluate oro-caecal transit time in cats before and after treatment for hyperthyroidism. *Can J Vet Res* 57:89-94, 1993.
- Solomons NW, Viteri FE, Hamilton LH: Application of a simple gas chromatographic technique for measuring breath hydrogen. *J Lab Clin Med* 90:856-862, 1977.
- Solomons NW, Garcia R, Schneider R, *et al*: H₂ breath tests during diarrhea. *Acta Paediatr Scand* 68:171-172, 1979.
- Spurrer JD: Two stage tests of hypothesis in the general linear model. *Communications in Statistics: Theory and Methods* 11:2775-2779, 1982.

Stevenson DK, Cohen RS, Ostrander CR, *et al*: A sensitive analytical apparatus for measuring hydrogen production rates. II. Application to studies in human infants. *J Pediatr Gastroenterol Nutr* 1:233-237, 1982.

Strocchi A, Levitt MD: Factors affecting hydrogen production and consumption by human fecal flora: the critical roles of hydrogen tension and methanogenesis. *J Clin Invest* 89:1304-1311, 1992a.

Strocchi A, Levitt MD: Maintaining intestinal H₂ balance: credit the colonic bacteria. *Gastroenterology* 102:1424-1426, 1992b.

Strocchi A, Ellis C, Levitt MD: Reproducibility of measurements of trace gas concentrations in expired air. *Gastroenterology* 101:175-179, 1991.

Thompson DG, Binfield P, De Belder A, *et al*: Extraintestinal influences on exhaled breath hydrogen measurements during the investigation of gastrointestinal disease. *Gut* 26:1349-1352, 1985.

Thompson DG, O'Brien JD, Hardie JM: Influence of the oropharyngeal microflora on the measurement of exhaled breath hydrogen. *Gastroenterology* 91:853-860, 1986.

Vogelsang H, Ferenci P, Frotz S, *et al*: Acidic colonic microclimate- possible reason for false negative hydrogen breath tests. *Gut* 29:21-26, 1988.

Washabau RJ, Strombeck DR, Buffington CA, Harrold D: Evaluation of intestinal carbohydrate malabsorption in the dog by pulmonary hydrogen gas excretion. *Am J Vet Res* 47:1402-1406, 1986a.

Washabau RJ, Strombeck DR, Buffington CA, Harrold D: Use of pulmonary hydrogen gas excretion to detect carbohydrate malabsorption in dogs. *J Am Vet Med Assoc* 189:674-679, 1986b.

CHAPTER 5

A COMPARISON OF WHEAT, POTATO, CORN AND RICE ASSIMILATION IN DOGS WITH DIARRHOEA USING BREATH HYDROGEN TESTS

Introduction

Several studies have compared the assimilation of different starches in healthy dogs (Moore *et al* 1980, Washabau *et al* 1986a, Schunemann *et al* 1990, Walker *et al* 1994). However, to the authors knowledge, the assimilation of starches in dogs with gastrointestinal disease have not been compared. This is surprising because it is possible that the factors limiting the assimilation of starch in healthy dogs may differ to the factors limiting starch assimilation in dogs with gastrointestinal disease. Animals with gastrointestinal disease frequently assimilate less carbohydrate than healthy animals. Hastened gastric emptying and/or orocolic transit times, reduced gastrointestinal secretions (α -amylase, bicarbonate), villus atrophy, microvillus damage, and bacterial overgrowth may all significantly alter the small intestinal assimilation of starch (Batt 1980, Chapman *et al* 1985, Williams and Guilford 1996). Despite these differences, it is often assumed that the relative digestibilities of different starches is the same in diseased and healthy dogs.

The breath H_2 test has been used in healthy people and in healthy dogs to compare the assimilation of different carbohydrates in the small bowel (Bond and Levitt 1972, Washabau *et al* 1986a, Anderson *et al* 1981, Levine and Levitt 1981, Levitt *et al* 1987). Although this technique can only be regarded as a semiquantitative measure of carbohydrate malabsorption (Strocchi and Levitt 1991), it is easily applicable to dogs in clinical situations, is simple to perform, and is a non-invasive procedure. However, the use of breath H_2 measurements to compare the assimilation of different types of carbohydrate is only meaningful if the carbohydrates tested release a similar amount of H_2 per gram of carbohydrate fermented, or if differences in their ability to produce H_2 are known. The objective of the present study was to use breath H_2 testing in conjunction with *in vitro* fermentation techniques to compare the assimilation of four different carbohydrate sources in dogs with gastrointestinal disease.

Materials and Methods

Animals

Twelve dogs that presented to the Massey University Veterinary Clinic with chronic diarrhoea due to maldigestion or malabsorption were used in this study. Seven of the dogs had maldigestion due to exocrine pancreatic insufficiency (EPI) and five dogs had malabsorption due to inflammatory bowel disease (IBD). The dogs were aged between 1 and 8 years, and weighed between 6 and 40 kg. All of the dogs with EPI were German Shepherds, whereas the dogs with IBD were of various breeds (1 Newfoundland, 3 Labradors, and 1 Chihuahua cross). The diagnosis of EPI was made on the basis of supportive clinical signs and a low serum trypsin-like immunoreactivity (TLI) assay ($< 2.5 \mu\text{g/l}$). The diagnostic criteria for IBD included: the presence of chronic "small bowel-type" diarrhoea of at least 2 weeks duration, negative faecal examinations for endoparasites, normal complete blood count, serum biochemistry profile, and serum TLI, and histologic abnormalities consistent with lymphocytic-plasmacytic (or eosinophilic) enteritis confirmed by at least two pathologists. Five of the dogs with IBD and two of the dogs with EPI were also investigated for small intestinal bacterial overgrowth (SIBO) via quantitative aerobic and anaerobic cultures of duodenal aspirates. The quantitative culture results were within our normal range ($< 3.7 \times 10^6$ aerobic, $< 1.3 \times 10^7$ anaerobic CFU/mL) for all of these cases. Owner consent was given for all tests performed and the experimental protocol was approved by the Massey University Animal Ethics Committee.

Study protocol

Four carbohydrate sources (wheat, potato, corn and rice flours) that are commonly included in commercial pet foods were tested in each dog separately. The nutritional profile of the carbohydrate sources are shown in Table 5.1. The carbohydrates were extrusion cooked^a and then mixed with raw ground beef for palatability. The carbohydrate and beef components of the meal were fed at a third MER and a quarter MER respectively (58% MER total). Daily MER were calculated by the formula $\text{kcal} = (\text{kg}^{0.75}) \times 132$. We have previously shown that the quantity of beef fed in this study produces minimal breath H_2 measurements over 18 hours after consumption (mean \pm SEM peak breath H_2 concentration of 2.11 ± 0.53 ppm).^b The dogs were fasted for 24 hours prior to feeding. The four meals were fed to all dogs in a balanced but

^a The Iams Company, Lewisburg, Ohio.

^b Bissett SA: unpublished data, 1996.

randomised order on days 1, 2, 4, and 5 of the test period. Day 3 consisted of a 24 hour rest period where the dogs were fed a commercial canine diet^c in the morning.

Table 5.1 Nutritional analysis of the wheat, potato, corn & rice flours

<i>Chemical composition (%DMB)</i>	<i>Wheat</i>	<i>Potato</i>	<i>Corn</i>	<i>Rice</i>
Protein	9.66	9.16	6.43	7.52
Ash	0.54	3.60	0.74	0.56
Fat	8.37	8.10	9.52	6.97
Crude fibre	1.85	1.14	0.93	0.00
Carbohydrate (by difference)	79.58	78.00	82.38	84.95

In vitro fermentation study^d

Substrates. Substrates used were wheat, potato, corn and rice flours. The chemical composition of the substrates are presented in Table 5.1.

Donors. Three dogs (two male and one female) had ad libitum access to a commercial dog food^c for 14 days before collection of a single faecal sample. The commercial diet fed contained beet pulp as a fibre source and met all nutrient requirements for dogs (NRC 1985). Immediately after defaecation (one person monitored dogs continuously), faeces were collected and transported in a sealed container to the laboratory for use in the *in vitro* fermentation procedure. Dogs had access to water at all times and were housed individually in a climate-controlled room with the temperature maintained at 20°C.

Medium composition and substrate fermentation. The composition of the medium used to culture the faecal microflora is presented in Table 5.2. All medium components, except the vitamin mix, was aseptically added after it was filter-sterilized. The freshly voided faecal sample from each dog was immediately placed in a plastic bag that was sealed after expressing excess air. Each sample was diluted approximately 1:10 in a 37°C anaerobic dilution solution (Bryant and Burkey 1953) by blending it for 15 seconds in a Waring blender. Blended, diluted faeces were filtered through four layers of cheesecloth in an anaerobic glove box.^e Appropriate sample and blank 20 ml headspace vials containing 6 ml of medium and 62 mg of substrate were aseptically inoculated with 200 µL of diluted faeces, resulting in a substrate concentration of 10 mg/ml. Tubes were capped with stoppers equipped with one-way gas release valves.

^c Eukanuba Premium, The Iams Company, Lewisburg, Ohio.

^d Performed at the Iams Technical Center, Lewisburg, Ohio.

^e LabConco, St Louis, Missouri.

Table 5.2 Composition of medium used to culture faecal microflora

<i>Component</i>	<i>Concentration in medium</i>
	(mL/L)
Solution A ^a	330.0
Solution B ^b	330.0
Trace mineral solution ^c	10.0
Water-soluble vitamin mix ^d	20.0
Folate:biotin solution ^e	5.0
Riboflavin solution ^f	5.0
Hemin solution ^g	2.5
Short-chain fatty acid mix ^h	0.4
Resazurin ⁱ	1.0
Distilled H ₂ O	296.0
	(g/L)
Yeast extract	0.5
Trypticase	0.5
Na ₂ CO ₃	4.0
Cysteine HCl.H ₂ O	0.5

^a Composition (g/L): NaCl, 5.4; KH₂PO₄, 2.7; CaCl₂.H₂O, 0.16; MgCl₂.6H₂O, 0.06; CoCl₂.6H₂O, 0.06; (NH₄)₂SO₄, 5.4.

^b Composition: K₂HPO₄, 2.7 g/L

^c Composition (mg/L): EDTA (disodium salt), 500; FeSO₄.7H₂O, 200; ZnSO₄.7H₂O, 10; MnCl₂.4H₂O, 3; H₃PO₄, 30; CoCl₂.6H₂O, 20; CuCl₂.2H₂O, 1; NiCl₂.6H₂O, 2; Na₂MoO₄.2H₂O, 3.

^d Composition (mg/L): thiamin.HCl, 100; pantothenic acid, 100; niacin, 100; pyridoxine, 100; p-aminobenzoic acid, 5; vitamin B₁₂, 0.25.

^e Composition (mg/L): folic acid, 10; biotin, 2; NH₄HCO₃, 100.

^f Composition: riboflavin 10 mg/L in 5 mmol/L HEPES.

^g Hemin, 500 mg/L in 10 mmol/L NaOH.

^h 250 mL/L each of n-valerate, isovalerate, isobutyrate, and DL- α -methylbutyrate.

ⁱ Resazurin, 1g/L in distilled H₂O.

Duplicate tubes were incubated at 37°C for 6, 12, and 24 hours. After the appropriate length of fermentation, 0.34 ml of 100% (w/v) metaphosphoric acid was added to each vial. All samples were then analysed within two hours. Concentrations of gas in the headspace of vials were determined with a HP68900 gas chromatograph fitted with a HP7694 headspace autosampler.^f Hydrogen and methane (CH₄) were separated on a Mol Sieve %A column^g at 30°C oven temperature with argon carrier gas flow at 3 ml/min. The column was connected to a

^f Hewlett Packard, Little Falls, Delaware.

^g 0.53 mm X 30 M, Supelco, Bellefonte, Pennsylvania.

thermal conductivity detector with a H₂ detection lower limit of 5 ppm. The analytes then proceeded through a transfer line to a flame ionisation detector where CH₄ was detected at a lower limit of 0.3 ppm (Leblanc *et al* 1993).

Breath collection and analysis

Breath samples were collected prior to feeding (baseline), and every 90 minutes for 18 hours after the test meals. See Chapter 3 for details regarding the collection and analysis of breath samples.

Statistical analysis

The GLM procedures of SAS (1988) were used to analyse both the breath H₂ and the *in vitro* fermentation data. The area under the breath H₂ concentration versus time curve (AUC) over the 18 hour test period was determined for each dog in each of the four treatment groups. The AUCs of the wheat, potato, corn, and rice meals were compared using repeated measures analysis of variance (ANOVA). Given that these meals were ingested over 4 consecutive days (except for the rest day) the effect of order of treatment was also assessed using ANOVA. In addition, ANOVA was used to compare the AUCs of the EPI and IBD dogs.

The *in vitro* fermentation experimental design was a randomised complete block with a 4 X 3 factorial arrangement of substrates and fermentation times as treatments. Dogs were used as the blocking factor in this experiment. The SAS model included the following terms: substrate, time, substrate X time, and donor. Arithmetic means are reported along with SEM for all treatments. When significant ($p < 0.05$) treatment effects were detected, means were compared by the least significant difference method (Cochran and Cox 1957).

Results

The mean \pm SEM breath H₂ concentrations of the twelve dogs after the ingestion of each carbohydrate source are presented in Figure 5.1. The mean \pm SEM fasting breath H₂ concentration of the twelve dogs determined from all four test days was 3.5 ± 1.1 ppm. None of the dogs demonstrated an early breath H₂ rise suggestive of SIBO.

The mean \pm SD AUC for the wheat, potato, corn, and rice meals were found to be 131.3 ± 97.9 ppm.h, 148.5 ± 112.9 ppm.h, 127.8 ± 90.0 ppm.h, and 122.4 ± 98.3 ppm.h respectively. No significant differences ($p = 0.64$) were demonstrated between the AUCs of the four carbohydrate meals. The order in which the four meals were fed to the dogs was also found

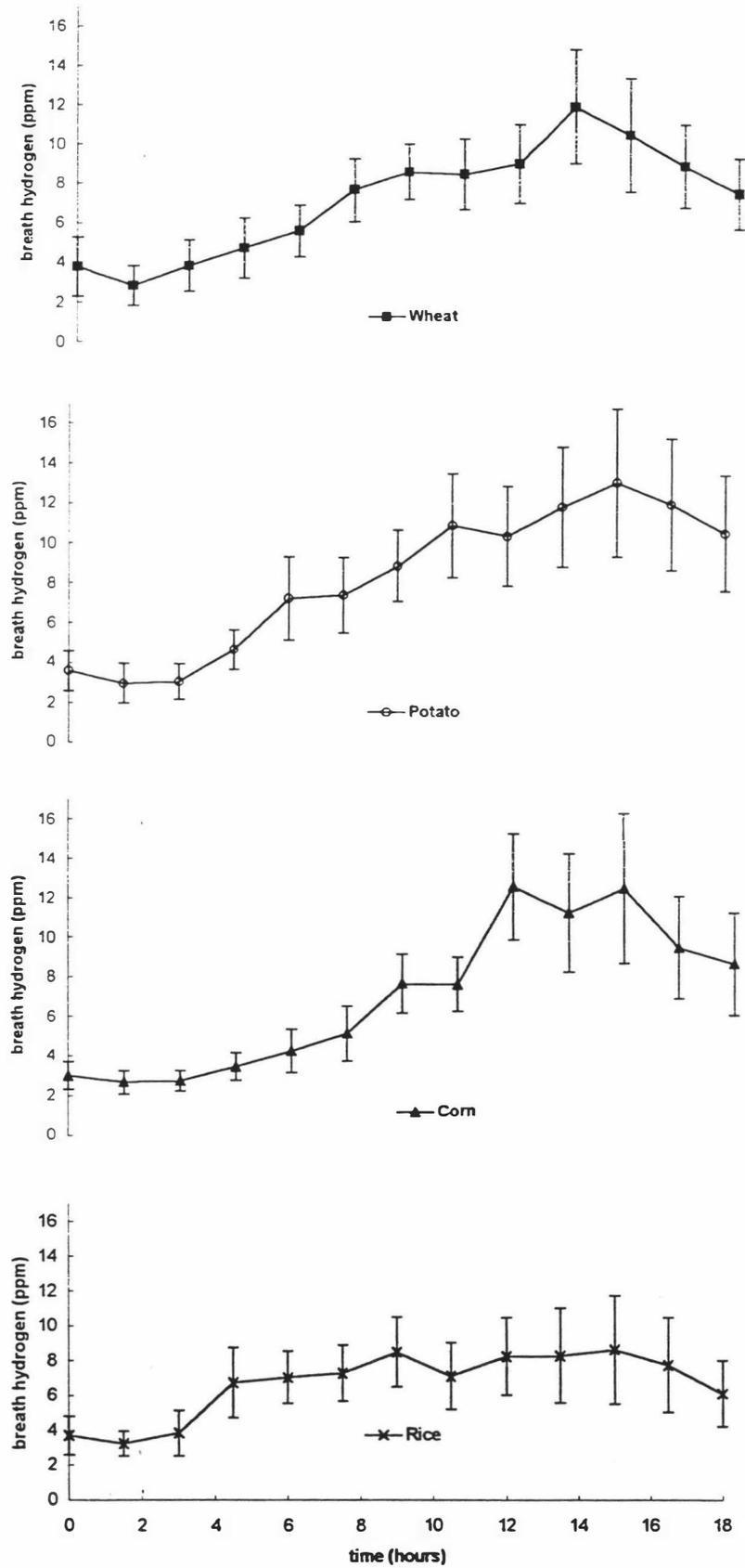


Figure 5.1 Mean \pm SEM breath H_2 concentrations of 12 dogs after the ingestion of wheat, potato, corn, and rice meals

to have no significant effect ($p = 0.63$) on the breath H_2 concentrations. Figure 5.2 displays the AUCs of each dog after the ingestion of the wheat, potato, corn and rice meals. Although there was no significant variation of breath H_2 concentrations between diets, variation between dogs was found to be highly significant ($p < 0.0001$). In addition, the interaction of dog by diet in the statistical analysis was also found to highly significant ($p < 0.0001$).

The amounts of H_2 and CH_4 produced per gram of carbohydrate after 6, 12 and 24 hours of *in vitro* fermentation with canine faecal flora are presented in Table 5.3. The four carbohydrate substrates appear to produce markedly different amounts of H_2 at each of the 3 different fermentation times, although the relationship of H_2 production between substrates is not the same for the different fermentation times. For example, after 6 hours of fermentation potato produced more H_2 than the other substrates (approximately 100% more than rice), while after 12 and 24 hours of fermentation corn produced more H_2 (> 50%) than the other sources. This observation is supported by a significant ($p = 0.03$) interaction of substrate by time for the H_2 data.

Table 5.3 Mean *in vitro* H_2 and CH_4 production of wheat, potato, corn and rice substrates

Substrate	Length of fermentation (hours)	H_2 ($\mu\text{mol}/\text{gram OM}^{\#}$ fermented)	CH_4 ($\eta\text{mol}/\text{gram OM}^{\#}$ fermented)
Wheat	6	431	2.5
	12	229	15.8
	24	223	32.4
Potato	6	532	1.7
	12	229	12.9
	24	168	24.1
Corn	6	468	0
	12	389	21.0
	24	367	32
Rice	6	268	0.3
	12	260	17.7
	24	220	27.5
SEM ^{##}		54.4	4.1

[#] organic matter; ^{##} standard error of the mean

When the dogs with EPI and IBD were considered separately, the AUCs for the different disease groups were found to be significantly greater ($p < 0.0001$) for the dogs with EPI (AUC \pm SD = 181.4 \pm 86.7 ppm.h) in comparison to the dogs with IBD (AUC \pm SD = 64.0 \pm 24.4 ppm.h). However, after repeating the ANOVA to compare the AUCs of the wheat,

potato, corn and rice for the EPI and IBD dogs separately, there was still found to be no significant effect of the type of starch fed ($p = 0.572$ for EPI dogs, and $p = 0.922$ for IBD dogs). Figure 5.3 displays the mean \pm SEM breath H_2 concentrations of the dogs with EPI and the dogs with IBD determined from data collected over all four test days.

Discussion

At “first glance”, the lack of significant variation between the AUCs of the different diets in this study suggests that the assimilation of wheat, potato, corn and rice in dogs with diarrhoea is similar. Unfortunately, this conclusion cannot be reached unless the amount of H_2 produced per gram of starch fermented is constant. Despite the finding that sugars, or sugars and purified starch, may produce similar quantities of H_2 per gram fermented (Bond and Levitt 1972, Carter *et al* 1981, Flourie *et al* 1988), recent studies have demonstrated that different carbohydrates are capable of producing variable amounts and types of fermentation gas (Levitt *et al* 1987, Grubler *et al* 1990, Christl *et al* 1992). In concordance with these studies, the *in vitro* fermentation results of the wheat, potato, corn and rice substrates in the present study demonstrate that marked differences (50-100%) exist between the amount of H_2 produced per gram of starch fermented by canine faecal flora after either 6, 12, or 24 hours of fermentation. This variation of H_2 production between the substrates, suggests that direct comparison of post prandial breath H_2 concentrations is not an accurate method of comparing the digestibility of different starches in dogs.

We gave consideration to “normalising” the breath H_2 curves against the *in vitro* fermentation data to compare the assimilation of the different carbohydrates. However, it is difficult to predict from the *in vitro* fermentation data which of the four carbohydrates would produce the most H_2 *in vivo*. For example, although potato produces the most H_2 after 6 hours of *in vitro* fermentation, potato produces the lowest amounts of H_2 after 12 and 24 hours of fermentation. Furthermore, significant H_2 consumption appears to be occurring after 6 hours in the fermentation system, as smaller H_2 quantities and larger CH_4 quantities are detected at 12 and 24 hours (Gibson *et al* 1990, Macfarlane and Cummings 1991, Christl *et al* 1992). Hydrogen consumption may be more prominent *in vitro* than *in vivo*, as H_2 accumulation in the closed *in vitro* system is likely to promote H_2 consumption. Because it is difficult to predict the duration over which carbohydrate fermentation would be occurring in the colon and the amount of H_2 consumption occurring *in vivo*, it is difficult to use these *in vitro* data to normalise the breath H_2 results.

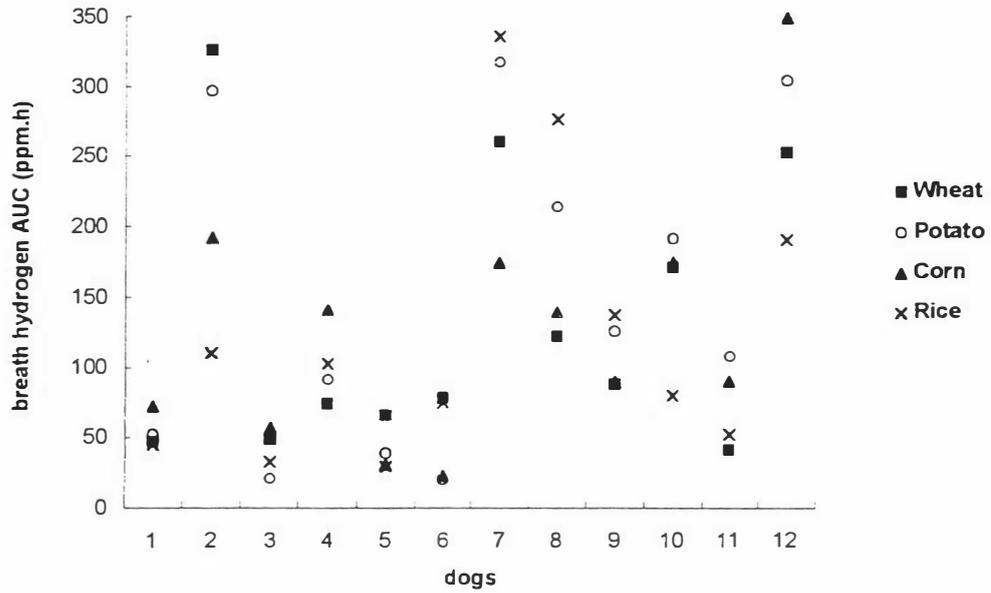


Figure 5.2 AUC data of the 12 dogs following each of the four different carbohydrate/beef meals

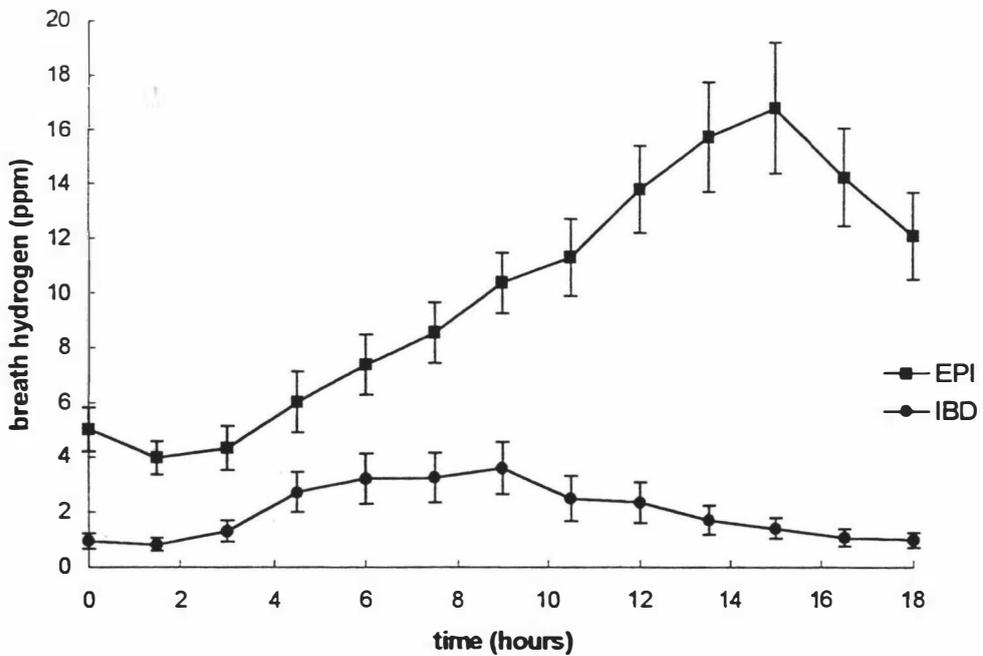


Figure 5.3 Mean \pm SEM breath H_2 concentrations of the dogs with exocrine pancreatic insufficiency (EPI) and inflammatory bowel disease (IBD)

Although the breath H_2 concentrations in this study did not vary significantly between the four meals tested, they were shown to vary significantly between dogs. Large individual variability in breath H_2 excretion after the ingestion of a test substrate has been reported in healthy dogs (Ludlow *et al* 1994), and is a well recognised phenomenon in people (Bond and Levitt 1972, Bjornekleit and Jenssen 1980, Flourie *et al* 1988, Rumessen *et al* 1990). The variation of breath H_2 concentrations between dogs in this study may be a reflection of individual variation, but may also be a consequence of different degrees of carbohydrate malassimilation (ie., different disease severity).

The interaction of dog by diet was also significant in this study. This finding suggests that the diets fed to the dogs *may* be affecting their breath H_2 concentrations, but that the effect of the diet is different for different dogs. For example, in Figure 5.2 it can be seen that rice results in the lowest breath H_2 concentrations in some of the dogs, but produces the greatest concentrations of breath H_2 in others. Therefore, dogs with gastrointestinal disease may vary considerably in their ability to assimilate different carbohydrates, which suggests that clinicians should consider feeding alternative carbohydrate sources to dogs with gastrointestinal disease that do not respond well to the initial diet fed. However, this finding cannot be confirmed, as the consistency of response of a given dog to a given carbohydrate was not assessed in this study.

The significant variation seen between the AUCs for the dogs with EPI and IBD confirm that the dogs with EPI in this study malabsorbed more of the carbohydrate test meals than the dogs with IBD. This finding is in contrast to Washabau's work, where dogs with small intestinal disease appeared to produce greater amounts of H_2 in the breath than dogs with EPI after the ingestion of xylose or a commercial canine diet (Washabau *et al* 1986b). However, differences in the severity of small intestinal disease between these studies is likely. The dogs with IBD in our study had only mild lymphocytic and plasmacytic infiltrates in their small intestine, and Washabau's study included dogs with small intestinal disease other than IBD.

In conclusion, the fermentation characteristics of complex carbohydrates in dogs appear to be variable, which compromises the value of the breath H_2 test as a method of comparing the assimilation of different carbohydrates. Individual dogs with gastrointestinal disease may vary in their ability to assimilate different carbohydrates. Finally, carbohydrate malabsorption in dogs with EPI is prominent, and exceeds the amount of carbohydrate malabsorbed by dogs that present with diarrhoea due to mild inflammatory bowel disease. Further work is needed to investigate carbohydrate assimilation and tolerance in dogs with gastrointestinal disease.

References

- Anderson IH, Levine AS, Levitt MD: Incomplete absorption of the carbohydrate in all-purpose wheat flour. *New Eng J Med* 304:891-892, 1981.
- Batt RM: The molecular basis of malabsorption. *J Small Anim Pract* 21:555-569, 1980.
- Bjorneklett A, Jenssen E: Measurement of pulmonary hydrogen (H₂) and H₂ diffusion from the small bowel and the colon. *Scand J Gastroenterol* 15:817-823, 1980.
- Bond JH, Levitt MD: Use of pulmonary hydrogen measurements to quantitate carbohydrate absorption. *J Clin Invest* 51:1219-1225, 1972.
- Bryant MP, Burkey LA: Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. *J Dairy Sci* 26:205, 1953.
- Carter EA, Bloch KJ, Cohen S, et al: Use of hydrogen gas analysis to assess intestinal absorption: studies in normal rats and in rats infected with the nematode, *Nippostrongylus brasiliensis*. *Gastroenterology* 81:1091-1097, 1981.
- Chapman RW, Sillery JK, Graham MM, Saunders DR: Absorption of starch by healthy ileostomates: effect of transit time and of carbohydrate load. *Am J Clin Nutr* 41:1244-1248, 1985.
- Christl SU, Murgatroyd PR, Gibson GR, Cummings JH: Production, metabolism, and excretion of hydrogen in the large intestine. *Gastroenterology* 102:1269-1277, 1992.
- Cochran WG, Cox GM: *Experimental Designs, 2nd Ed.* John Wiley and Sons, New York, 1957.
- Flourie B, Florent C, Etanchaud F, et al: Starch absorption by healthy man evaluated by lactulose hydrogen breath test. *Am J Clin Nutr* 47:61-66, 1988.
- Gibson GR, Cummings JH, Macfarlane GT, et al: Alternative pathways for hydrogen disposal during fermentation in the human colon. *Gut* 31:679-683, 1990.
- Grubler B, Ingwersen M, Meyer H, Schunemann C: In vitro investigations on gas formation from ileum and colon chyme in dogs. *Nutrition, Malnutrition and Dietetics in the Dog and Cat.* 34-36, 1990.
- Leblanc YR, Gilbert M, Duval M, Hubert J: Static headspace determination of fault gases dissolved in transformer insulating oils. *J Chromatography* 633:185-193, 1993.
- Levine AS, Levitt MD: Malabsorption of starch moiety of oats, corn and potatoes. *Gastroenterology* 80:1209, 1981.
- Levitt MD, Hirsh P, Fetzer CA, Sheahan M, Levine AS: H₂ excretion after ingestion of complex carbohydrates. *Gastroenterology* 92:383-389, 1987.
- Ludlow CL, Bruvette DS, Davenport DJ, Lieth DE: Daily and individual variation of breath hydrogen excretion in healthy dogs. *J Vet Int Med* 8:150, 1994.

Macfarlane GT, Cummings JH: The colonic flora, fermentation, and large bowel digestive function. In: Phillips SF, Pemberton JH, Shorter RG (eds) *The Large Intestine: Physiology, Pathophysiology, and Disease*. Raven Press, New York, 51-92, 1991.

Moore ML, Fottler HJ, Fahey GC, Corbin JE: Utilization of corn-soyabean meal-substituted diets by dogs. *J Animal Sci* 50:892-896, 1980.

NRC: *Nutrient Requirements of Dogs*. National Academy Press, Washington DC, 1985.

Rumessen JJ, Hamberg O, Gudmand-Hoyer E: Interval sampling of end-expiratory hydrogen (H₂) concentrations to quantify carbohydrate malabsorption by means of lactulose standards. *Gut* 31:37-42, 1990.

SAS: SAS/STAT® User's Guide (release 6.03). SAS Inst. Inc., Cary, NC, 1988.

Schunemann C, Muhlum A, Meyer H: Precaecal and post ileal digestibility of various carbohydrates in dogs. *Nutrition, Malnutrition and Dietetics in the Dog and Cat* 23-26, 1990.

Solomons NW, Viteri FE, Hamilton LH: Application of a simple gas chromatographic technique for measuring breath hydrogen. *J Lab Clin Med* 90:856-862, 1977.

Strocchi A, Levitt MD: Measurement of starch absorption in humans. *Can J Physiol Pharmacol* 69:108-110, 1991.

Walker JA, Harmon DL, Gross KL, Collings GF: Evaluation of nutrient utilization in the canine using the ileal cannulation technique. *J Nutr* 124:2672S-2676S, 1994.

Washabau RJ, Strombeck DR, Buffington CA, Harrold D: Evaluation of intestinal carbohydrate malabsorption in the dog by pulmonary hydrogen gas excretion. *Am J Vet Res* 47:1402-1406, 1986a.

Washabau RJ, Strombeck DR, Buffington CA, Harrold D: Use of pulmonary hydrogen gas excretion to detect carbohydrate malabsorption in dogs. *J Am Vet Med Assoc* 189:674-679, 1986b.

Williams DA, Guilford WG: Malabsorption, small intestinal bacterial overgrowth, and protein-losing enteropathy. In: Guilford WG, Center SA, Strombeck DR, Williams DA, Meyer DJ (eds) *Strombeck's Small Animal Gastroenterology, 3rd ed.* W.B. Saunders, Philadelphia. 1996, pp 367-380.

CHAPTER 6

CONCLUSIONS

Breath H₂ tests are used in humans for a variety of diagnostic purposes, to investigate the pathophysiology of gastrointestinal symptoms, and to study the nutritional properties of carbohydrates. Only a limited number of studies have applied breath H₂ tests to animals over the past decade. This thesis has investigated the use and role of breath H₂ testing in the study of carbohydrate malabsorption in dogs. In particular, carbohydrate malabsorption has been assessed in healthy dogs and dogs with gastrointestinal disease to investigate the effects of carbohydrate processing and of feeding different carbohydrate sources. These studies were conducted with the aim to further our knowledge of carbohydrate digestion in dogs, and to provide recommendations on the nutritional management of gastrointestinal disorders. In addition, the effect of 5% dehydration on breath H₂ measurements in dogs was determined to assess if hydration status is an important variable affecting breath H₂ tests when they are used for diagnostic purposes in clinical practice.

Significant carbohydrate malabsorption may occur in dogs due to a variety of gastrointestinal disturbances which interfere with the “normal” digestion and absorption of carbohydrates in the small intestine. Minimising carbohydrate malabsorption can be achieved by feeding highly digestible diets, and such diets are often included in the therapy of gastrointestinal disorders to reduce the clinical signs associated with carbohydrate intolerance. Breath H₂ tests performed on healthy dogs in this study, have demonstrated that the reduction of food particle size may improve carbohydrate digestibility and may also alter a carbohydrate’s orocolic transit time and/or fermentability. Although only a small number of studies have used breath H₂ tests to investigate the effects of food processing on carbohydrate assimilation in humans, our findings demonstrate that breath H₂ tests can provide valuable information regarding the gastrointestinal handling of dietary carbohydrates in dogs. An opportunity exists to more widely apply breath H₂ testing to animals in the study of carbohydrate nutrition, particularly to dogs and cats in which studies of carbohydrate digestion are scarce. The technique is non-invasive, well tolerated, and inexpensive, and is therefore particularly suited for use in veterinary studies.

Breath H₂ tests have been used in the past to compare the assimilation of *different* sources of carbohydrates fed to humans and dogs. This has been achieved by comparing breath H₂ concentrations after feeding the same quantities of different carbohydrates. However, this method of comparing the assimilation of different carbohydrates assumes that similar amounts

of H₂ are produced per gram of substrate fermented. We attempted to compare the assimilation of extrusion cooked wheat, potato, corn and rice flours in dogs with diarrhoea using breath H₂ tests. *In vitro* fermentation of the four carbohydrate sources was performed to determine if there were significant differences in the H₂ producing ability of these carbohydrates. The *in vitro* studies revealed large variations in the amount of H₂ produced per gram of substrate fermented at different fermentation times. This variation of H₂ production did not allow us to use the breath H₂ concentrations to compare the assimilation of the four carbohydrates. However, breath H₂ studies may still be useful for comparing the assimilation of different carbohydrates if an accurate method of comparing the *in vivo* H₂ production of different carbohydrates is developed. For example, *in vitro* fermentation studies using proximal colonic microflora and fermentation systems that allow gas to be continuously withdrawn from the system may more accurately predict and quantitate *in vivo* H₂ production.

Although we were unable to accurately compare the assimilation of wheat, potato, corn and rice flours in dogs with gastrointestinal disease, some interesting observations were made from this experiment. Breath H₂ concentrations appeared to vary markedly for different diets within individual dogs. Individual variation of carbohydrate assimilation suggests that a single carbohydrate source can not be predicted to be the "preferred" or "most digestible" carbohydrate source in all dogs with gastrointestinal disease. In addition, carbohydrate malabsorption was shown to be prominent in dogs with exocrine pancreatic insufficiency, and occurred to a greater degree than in dogs with mild inflammatory bowel disease. This finding emphasises the importance of feeding diets containing moderate amounts of highly digestible carbohydrates to dogs with exocrine pancreatic insufficiency. Further investigations regarding the assimilation and tolerance of different carbohydrate sources is warranted in dogs with gastrointestinal disease, particularly with respect to identifying whether a "preferable" source of carbohydrate exists. Alternative methods of investigating carbohydrate malassimilation in humans include ileal intubation techniques and ileostomy models. Although these methods are more invasive than breath H₂ testing, their application to dogs with gastrointestinal disease would provide valuable information on carbohydrate digestion in diseased animals.

Breath H₂ testing can be used as a diagnostic test for a variety of gastrointestinal disorders in humans and animals. In veterinary clinical practice, breath H₂ tests are well suited for use as diagnostic tests for carbohydrate malabsorption, small intestinal bacterial overgrowth, and for estimating orocolic transit times. Unfortunately, numerous variables may influence the amount of H₂ excreted in the breath and cause problems with the interpretation of test results. We determined the effect of 5% dehydration on breath H₂ concentrations in dogs, as breath H₂ tests are often performed on dogs with gastrointestinal disorders which are associated with the

loss of body fluids. Mild dehydration was found to elevate breath H_2 concentrations. In addition, we discovered that dogs may pass flatus during the collection of breath samples resulting in falsely elevated breath H_2 readings. We observed that this most commonly occurs during peak breath H_2 readings, as excessive flatulence is also likely to occur at these times. Hydrogen gas is not odiferous unless accompanied by other gases, and therefore the passage of flatulence cannot always be detected by smell. However, large differences in H_2 concentrations between duplicate samples (usually in excess of 100%) and inappropriate increases in breath H_2 concentration in relation to the preceding and following values, usually allows easy identification of contamination of ambient air by flatus. Mild dehydration was associated with greater numbers of flatus-contaminated breath samples. The large number of flatus-contaminated breath samples and elevated breath H_2 concentrations detected in the dehydrated dogs, suggests that suboptimal hydration results in a total increase in the “net” amount of H_2 produced in the gastrointestinal tract. Therefore, consideration needs to be given to the hydration status of an animal prior to breath H_2 testing to increase the specificity of the breath H_2 test. Before breath H_2 tests can be widely applied in veterinary clinical practice as diagnostic tests, further work is needed to develop accurate reference ranges, to examine the reproducibility of breath H_2 tests, and to determine the most suitable test substrate for use in animals.

In conclusion, this thesis has demonstrated that breath H_2 testing can be a valuable research tool for investigating the nutritional properties of carbohydrates in animals. However, researchers must be aware of the limitations of this technique, particularly with regard to comparing the assimilation of different carbohydrate sources which may vary in their fermentability characteristics. Breath H_2 tests are also particularly well suited as diagnostic tests for veterinary clinical practice. Further work in this area will help to clarify the diagnostic accuracy of breath H_2 tests and determine their role in small animal practice.