

The work presented in this Thesis and the compilation of the Thesis,  
unless otherwise acknowledged are my own.

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## ABSTRACT

Anaerobic bacterial metabolism in the colon produces hydrogen, methane, volatile fatty acids and carbon dioxide. Hydrogen and methane are excreted in flatus and the expired breath. Investigations in this Thesis attempted to identify factors which may influence methane excretion in man and explain why all subjects do not excrete methane. The proportions of methane producing subjects in two healthy populations studied in Edinburgh were 33% and 70% respectively. Age and sex did not significantly alter methane excretion.

Dietary intakes, faecal components and bowel function were found to be similar in methane producing and non-methane producing subjects. An association was found between the ingestion of the pentose fraction of non-cellulosic polysaccharides and the concentration of breath methane in methane producing subjects. This association may be the result of a steady metabolic state in the caecum. Ingestion of pentose monomers D(+) xylose and L(+) arabinose increased methane excretion, in methane producing subjects, over five hours of study. The lack of gas production following acute complex polysaccharide administration could be due to a relatively slow metabolic response of colonic bacteria. Production of methane following the acute administration of free pentoses and the absence of methane production after the ingestion of polysaccharide sources may indicate that the release and availability of free pentose monomers from plant polysaccharides may be rate limiting steps in this process.

Patient groups with clinically defined diseases appeared to have altered prevalences of methane production compared to control populations. In healthy populations there is great variation in the proportion of

methane producers and the concentrations excreted. It is therefore unlikely that the methane status of an individual could be used as a diagnostic aid. Results of methane status may only be significant in population studies.

The inaccessibility of the human caecum necessitates the use of animal models and in vitro bacterial cultures. Caecectomy and the feeding of an elemental diet to intact rats abolished methanogenesis. A gum arabic supplemented control diet increased methanogenesis whereas methane excretion remained absent when gum arabic was added to the elemental diet. Methanogenic bacteria appear to colonise the caecum and require a fibrous residue or matrix for colonisation and as a substrate. Small but significant amounts of methane were produced by Clostridium histolyticum, C. perfringens and C. septicum in pure culture. Simple in vitro experiments with the addition of various substrates to the growth medium of two control methanogens, Methanobrevibacter ruminantium and Methanosarcina barkeri and C. histolyticum gave a confusing pattern of results, however L(+) arabinose increased gas production from each of the bacteria. Human methane production may result from the metabolism of other gastrointestinal organisms such as Clostridia.

## CONTENTS

	<u>Page</u>	
Chapter 1	Introduction	1
Chapter 2	Subjects and Methods	20
	1. Participants	20
	2. Gas sampling techniques	21
	3. Method of gas analysis	23
	4. Diet analysis	25
	5. Faecal component analysis	27
	6. Intestinal transit time and caecal retention time measurements	29
	7. Histology	31
	8. Statistics	32
Chapter 3	The prevalence of breath methane excretion in normal populations	35
Chapter 4	The influence of pentose on breath methane excretion	55
Chapter 5	The prevalence of breath methane excretion in clinical populations	62
	1. Gastrointestinal patients	64
	2. Geriatric patients	74
	3. Atherosclerosis patients	86
Chapter 6	The rat - an animal model	88
	1. Effect of age and sex on methane excretion	89
	2. Caecectomy and arterial ligation experiments.	91
	3. Effect of elemental diet on methane excretion	91
	4. Gum acacia supplementation	93
	5. High fibre, Spratt's/bran and low fibre, meat/egg diets	96
Chapter 7	Methane and hydrogen production by anaerobic bacteria of the human large intestine	102
Chapter 8	Discussion	108
	Conclusions and future areas of research	132
	References	136
	Appendix	
	1. Clinical details of patients in Chapter 5	147
	2. Description of chemicals used	157

## INDEX OF TABLES

<u>Table</u>	<u>Title</u>	<u>Page</u>
1	Characteristics and nutritional requirements of <u>Methanobacterium ruminantium</u> and <u>Methanosarcina barkeri</u>	6
2a	Chromatographic working conditions	33
b	Spratt's Laboratory Small Animal Diet	33
c	Components of wheat bran	34
d	Flexical complete elemental diet	34
e	Rat biscuit diet - Oxoid Breeding diet	34
3a	Number of methane producers, mean and standard deviation methane concentration for group 1 (hospital staff and students)	40
b	Number of methane producers, mean and standard deviation methane concentration for group 2 (local volunteers)	40
c	Mean, standard deviation and coefficient of variation of hourly and daily methane concentrations	42
d	Relation between breath methane concentration ( $\mu\text{mol/l}$ ) and respiratory excretion rate ( $\mu\text{mol/min}$ )	45
e	Daily dietary intakes for group 1 subjects	46
f	Daily dietary intakes for group 2 subjects	46
g	Dietary intakes for methane producers and non-producers in group 1 subjects	47
h	Daily dietary intakes for methane producers and non-producers in group 2 subjects	48
i	Daily dietary intakes of nutrition experiment subjects	50
j	Details of faecal components and bowel function for group 1 subjects	52
k	Details of faecal components and bowel function for group 2 subjects	52
l	Details of faecal components and bowel function for methane producers and non-producers in group 2 subjects	53
m	Details of faecal components and bowel function for nutrition experiment subjects	54

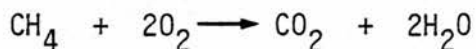
<u>Table</u>	<u>Title</u>	<u>Page</u>
4a	Formulae of pentoses and xylan	57
b	Base line, mean and standard deviation breath methane over a 5 hour period at 30 minute intervals in controls (fasting) and after ingestion of test meals	60
5a	Diagnostic criteria	65
b	Breath methane and hydrogen concentrations for gastrointestinal and control patients	67
c	Base line, mean and standard deviation breath hydrogen and methane concentrations following a glucose test meal	70
d	Base line, mean and standard deviation breath hydrogen and methane concentrations following a lactose test meal	71
e	Methane excretion in geriatric patients	77
f	Mean of the total number of pellets in the caecum/ascending colon and the descending colon/rectum on days 2, 3 and 4	85
g	Methane excretion in atherosclerosis patients	87
6a	Effect of arterial ligation on methane and hydrogen excretion (mls/kg.hr)	92
b	Hydrogen and methane excretion in rats on a gum acacia supplemented diet	94
c	Increase in volatile fatty acid (mg/g faeces) over 4 weeks for test and control rats	95
d	Faecal volatile fatty acids in 3 week old rats (mg/g)	98
e	Faecal volatile fatty acids in 14 week old rats (mg/g)	99
f	Faecal and caecal volatile fatty acids in 14 week old rats (mg/g)	100
7a	Methane and hydrogen concentrations ( $\mu\text{mol/l}$ ) in headspace gas from pure bacterial cultures	105
b	Methane and hydrogen concentrations ( $\mu\text{mol/l}$ ) in headspace gas after incubation of test strain with carbohydrate substrates	107

## INDEX OF FIGURES

<u>Figure</u>		<u>Page</u>
2a	Chromatogram of breath and standard gas samples using a katharometer detector	24
b	Chromatogram of rat, room air and standard gas samples using a flame ionization detector	26
c	Chromatogram of volatile fatty acids using a flame ionization detector	30
3a	Breath methane concentrations ( $\mu\text{mol/l}$ ) in healthy subjects	41
b	Daily variations in methane concentration ( $\mu\text{mol/l}$ ) over 4 weeks in 3 subjects	43
c	Relationship between breath methane concentration ( $\mu\text{mol/l}$ ) and daily intake of pentose (mmols) in 34 subjects	49
4a	Base line, 2, 3, 4 and 5 hour breath methane concentrations ( $\mu\text{mol/l}$ ) after a control period of fasting and following the ingestion of D(+) xylose and L(+) arabinose	61
5a	Breath methane concentrations ( $\mu\text{mol/l}$ ) in clinical patients	66
b	Breath hydrogen and methane concentrations ( $\mu\text{mol/l}$ ) following a 50g glucose test meal in one typical subject	72
c	Breath hydrogen and methane concentrations ( $\mu\text{mol/l}$ ) following a 50g lactose test meal in one typical subject	73
d	Abdominal X-ray of a patient with a faeces-filled caecum	75
e	Abdominal X-ray of a patient with a gas-filled caecum	76
fi, ii, iii	Radiography of the same subject with a faeces-filled caecum on days 2, 3 and 4 after swallowing 20 radio-opaque markers on days 1, 2 and 3	79, 80, 81
gi, ii, iii	Radiography of the same subject with a gas-filled caecum on days 2, 3 and 4 after swallowing 20 radio-opaque markers on days 1, 2 and 3	82, 83, 84
6a	Growth curves of male and female Wistar rats	90

CHAPTER 1  
INTRODUCTION

Methane, marsh gas or fire damp is the first hydrocarbon of the paraffin series. It is an alkane which is a saturated aliphatic hydrocarbon. The name methane is derived from the name of the one-carbon alcohol, methyl alcohol, which can be obtained from wood. Its name comes from the Greek 'methy', wine, and 'yle', wood. Methane is an odourless, inflammable gas with a boiling point of  $-161.5^{\circ}$ , which forms an explosive mixture with air. Coal gas and natural gas have methane as a major constituent. Natural gas is frequently found along with liquid petroleum deposits. Combustion of methane results in the cleavage of the carbon-carbon and carbon-hydrogen bonds. Complete combustion results in the formation of carbon dioxide and water. (Watson et al., 1978).



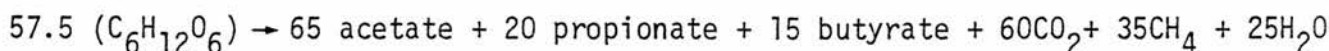
Methane is formed from decaying organic matter in marine and freshwater sediments. Occasionally methane has been ignited over marshes by lightning which has led to the name "will'-o'-the-wisp". In 1776 the Italian physicist Alessandro Volta (1745 - 1827) discovered the formation of "combustible air" in the sediments of streams, bogs, and lakes rich in decaying vegetation. Flammable gases that are sometimes trapped within the trunks of living trees contain methane. This is associated with an abnormal condition of heartwood known as wetwood which is an infestation of anaerobic bacteria (Zeikus, 1977). Methane found in coalmines is believed to have been trapped there when the coal was formed from carboniferous swamps. A low concentration of methane (1.65 parts per million) is present in the atmosphere (Ehhalt, 1979). Atmospheric methane originates mainly from ruminants, swamps and paddy fields (Vogels, 1979).

Methane is commonly produced in the digestive tract of animals, especially the rumen. In non-ruminants the caecum is an organ in which a similar anaerobic digestion may take place. Methane has also been obtained from the caeca of birds (Gasaway, 1976) and from some invertebrates (Donelan, 1974).

Sludge fermenters of sewage plants generate vast quantities of methane which may be used to operate diesel engines and to generate electricity.

Interest in methane has developed from several different areas including ruminology, fuel production from anaerobic digestion processes and manned space flights.

In the rumen a population of about  $10^{10}$  bacteria and  $10^6$  protozoa hydrolyse cellulose and other plant structural polysaccharides and ferment sugars. Volatile fatty acids, mainly acetic, propionic and butyric acids, together with methane and carbon dioxide are end products. Hydrogen, formate, succinate and lactate are not rumen end products since they are further metabolised. Formate is rapidly degraded to carbon dioxide and hydrogen is utilised in methane production. Volatile fatty acids are absorbed through the rumen wall and are utilised by the animal as a major source of energy, (Bauchop 1977). Formation of methane is considered to be a wasteful process resulting in approximately 8 to 10 per cent of the gross energy of the animal's diet being excreted. The following equation is a typical balance for the fermentation of carbohydrate in the rumen (Wolin, 1960).



A large ruminant may produce 200 or more litres of methane per day which is removed by belching. It was thought to be of value to include methane inhibitors in cattle diets to overcome the energy loss.

By this it was reasoned that energy retention would be increased. These inhibitors were polyunsaturated fatty acids of halogenated methane analogues (alkyl halides). However, there were several problems associated with the inhibitors including a diminished appetite and an adaptation of the flora to the inhibitors (Prins 1978). Inhibition of methanogenesis led to an accumulation of hydrogen and increased rates of propionate production. Recently, antibiotics monensin and rumensin have been introduced as feed additives to stimulate propionate formation directly, affecting methane production indirectly, (Thornton and Owens, 1981).

As fossil fuels are depleted and concern for the environment grows, biological energy production processes are assuming greater importance. Biomass containing a high moisture content, for example, algae, most field crops and livestock waste can be fermented and yield methane. This process is very versatile and can be used in large scale urban sewage treatment plants of over 3000m<sup>3</sup> tank capacity, or in small scale systems, the 3 to 4m<sup>3</sup> Gobar vessels of rural India. Anaerobic digestion can increase the energy output of livestock waste sixfold and provides an improved fertiliser over composted manure, (Lewis 1980). This reaction has two separate phases. The first phase involves the breakdown of large polymers like cellulose to soluble organic compounds and eventually acetic and propionic acids by non-methanogenic bacteria. Methane is produced by methanogenic bacteria in the second phase. Methanogenic bacteria were isolated from sewage sludge by Smith (1966).

Interest in methane production in man has arisen with the development of space travel. The accumulation of flatus, with high concentrations of hydrogen and methane, in a confined space could create

an explosive hazard. Gas chromatographic measurements of breath hydrogen and methane were applied as part of the investigations into the effects and the role of intestinal gas in manned space flight, (Calloway and Murphy, 1968). This work was discussed at a conference in Florida on Nutrition in space and related waste problems (Murphy 1964).

The bean industry sponsored research into the origin and nature of bean-related flatulence. Beans were found to be rich in oligosaccharides, for example stachyose and raffinose which are fermented by the colonic flora (Steggerda 1968). The gas-forming potential of a variety of fruits, legumes and cereals has been investigated, (Calloway 1966; Hickey et al., 1972). Hydrogen production was found to be substrate related but the factors influencing methane production were not identified. In vitro fermentations of faecal or ileo-dejecta flora with various substrates produced high concentrations of hydrogen with carbohydrates. Methane production was increased when amino acid substrates were used (Calloway et al., 1966).

Applications of breath hydrogen measurements have been many and varied (Newman 1974). Several clinical applications are the diagnosis of disaccharidase deficiencies (Calloway and Murphy 1968; Metz et al., 1976a; Newcomer et al., 1977), small intestinal bacterial colonisation (Metz et al., 1976b), pneumatosis cystoides intestinalis (Gillon et al., 1978; Van der Linden and Marsell 1979) and the measurement of small intestinal transit time (Bond and Levitt 1975; Metz et al., 1976c).

Breath methane measurements are not used as yet for any routine diagnostic aid. A recent finding that 80 per cent of patients with colonic carcinoma excrete breath methane compared to 40 per cent of

control patients (Haines et al., 1977) has stimulated further research into potential applications of breath methane measurements. Studies into bacterial activity in the gut and the metabolism of dietary fibre in the colon have involved breath hydrogen and methane analysis, (Gilat et al., 1978; Tadesse and Eastwood 1978).

For many decades the investigation of methanogens was modest both by microbiological and biochemical techniques largely because of difficulties of culture. Methanogenic bacteria are the strictest anaerobes known, those from the gut requiring a redox potential below  $-0.33V$  at pH7 to initiate growth (Smith and Hungate 1958). Several workers developed specialised culture techniques for the anaerobic methanogens. In 1940 H.A. Barker developed the pyrogallol carbonate tube. The Hungate roll tube and broth tube were developed by RE Hungate (1950, 1969). An atmosphere of 80 per cent hydrogen and 20 per cent carbon dioxide at 2 atmospheres pressure was incorporated into the method of Balch and Wolfe (1976). Growth of methanogens in Petri dishes was achieved in an Ultra Low Oxygen Chamber(ULOC) by Edwards and McBride (1975). An anaerobic glove box for the isolation and growth of methanogenic bacteria has been described by Cox and Herbert (1978). The principles and applications of the various anaerobic techniques are discussed by Hutton (1980). There are several reviews of the characteristics and classification of methanogens (Zeikus 1977; Woese and Fox 1978; Balch et al., 1979; Wolfe 1979) and the formation of methane (Stadtman 1967; Wolfe 1971; Mah et al., 1977; Wise et al., 1978; Wolfe and Higgins 1979).

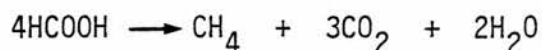
Methanogens are a morphologically diverse group of organisms which share the common metabolic capacity to produce methane. Of the three genera proposed to have this metabolic characteristic in the most recent

edition of Bergey's Manual of Determinative Bacteriology (1974) only one genus is moderately well characterised and contains more than one species that is available in pure culture. This genus, Methanobacterium, contains both gram-negative and gram-positive cells and the morphologies vary from short cocci to long, filamentous rods. Three species of this genus have been found in the gut - M. formicicum, M. ruminantium and M. mobile (Clarke 1977). M. ruminantium, the most common species in ruminants has been isolated from human faeces (Nottingham and Hungate 1968). In 1970 only 8 species of methanogen were available in pure culture. The following table contains details of 2 of these bacteria, Methanobacterium ruminantium isolated from the rumen and Methanosarcina barkeri isolated from mud and sludge.

CHARACTERISTICS AND NUTRITIONAL REQUIREMENTS OF  
METHANOBACTERIUM RUMINANTIUM AND METHANOSARCINA BARKERI  
(FROM WOLFE 1971)

	<u>Methanobacterium ruminantium</u>	<u>Methanosarcina barkeri</u>
Source	Rumen and sludge	mud and sludge
Morphology	Coccus to short rod in chains	Sarcina
Gram reaction	+	+
Substrates	Hydrogen, carbon dioxide, formate	Hydrogen, carbon dioxide, methanol, acetate
Nutritional requirements		
Nitrogen	Ammonia	Ammonia
Vitamins	Growth factor from rumen fluid (B vitamins stimulatory to bacteria from sludge)	None
Other	Acetate; 2methyl-butyrate	None

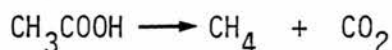
With the exception of M. barkeri, the preferred substrates are either hydrogen and carbon dioxide or formate. Ammonium ions are the main source of nitrogen. Acetate is stimulatory for many species and 2-methyl butyrate is a source of branch-chain carbon skeletons for certain species. M. ruminantium is a formate-fermenting species. The fermentation catalysed by this species is described by the following equation (Stadtman 1967).



Mixtures of carbon dioxide and hydrogen can be converted to methane according to the following equation



M. barkeri is an acetate-fermenting species



which can also ferment methanol



Production of carbon dioxide and methane is a unique property of methanogens. Wolfe (1979) describes several other characteristics unique to methanogens which include their narrow range of substrates, their requirement for a low redox potential and the presence of seven new coenzymes or factors. Methanogenic bacteria are representative of a group of organisms, the archaebacteria (Woese and Fox, 1978). Genealogically they bear no more relationship to other bacteria than they do to eukaryotes. The authors conclude that archaebacteria and typical bacteria represent separate lines of descent.

Methane production has been extensively investigated in ruminants. The rumen is anatomically complex, consisting of four chambers. Only the true stomach or abomasum has peptic glands and the food is fermented by micro-organisms before it reaches the acid stomach. The rumen and

reticulum together form the fermentation chamber. The omasum has thin page-like folds of epithelium so that digesta passing through are close to an absorption surface. The abomasum or acid stomach is the fourth chamber. Rate of passage of digesta from the rumen is characteristically slow and is a major factor in the degree of cellulose digestion. The adult rumen accounts for 80 per cent of the total stomach capacity. In feeding, grass and forage pass first from the oesophagus into the reticulum where they are made into a small ball of cud. This is returned to the mouth and chewed again. When reswallowed the food enters the rumen where it is bacterially digested.

Although it has been known since the 1880's that ruminal fermentation produces mainly acetic, propionic and butyric acids, knowledge of the carbohydrate metabolism of ruminants remained confused until 1944 when Barcroft et al., showed that these acids were readily absorbed and metabolised.

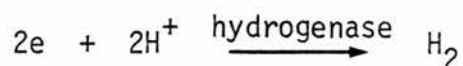
Formation of methane can be used as an index of fermentation rates. The type and amount of diet affect methane production, (Hungate 1968). Carbohydrates, which are the chief substrates, consist of complex cell wall polysaccharides such as cellulose, hemicellulose and pectin, storage polysaccharides such as starch and sugars which are digested by microbial enzymes. Pentoses are converted to hexoses which are fermented by the glycolytic pathway. In the conversion of pyruvate to acetyl CoA hydrogen gas or formate is formed which together with carbon dioxide are the substrates for methanogenesis in the rumen.

The rumen contains a diverse bacterial population. Gram-negative bacteria are species of Bacteroides, Butyrivibrio, Veillonella, Selenomonas, Treponema, Succinivibrio, Succinimonas, Anaerovibrio, Megasphaera, and

Acholeplasma. Gram-positive genera represented are Ruminococcus, Eubacterium, Lachnospira, Methanobacterium, Streptococcus, Propionibacterium, Lactobacillus and Clostridium. Bacterial floras of non-rumen alimentary microbial ecosystems (except that in human faeces) have not been studied as thoroughly as those in the rumen (Hungate 1976).

The most recent developments in the study of rumen methanogenesis have come from studies on hydrogen transfer between methanogenic and non-methanogenic bacterial species (Mah et al., 1977; Prins 1979). Interspecies hydrogen transfer allows methanogenic bacteria to obtain hydrogen and carbon dioxide from substrates such as carbohydrates, proteins or fatty acids which they are otherwise incapable of using. This transfer of hydrogen occurs when the flow of fermentation generated electrons is shifted from the fermentation of reduced organic end products for example propionate or ethanol, to proton reduction. Continuous use of hydrogen by methanogenic bacteria or other hydrogen-utilising bacteria and maintenance of a low partial pressure of hydrogen allows the hydrogen producing bacteria to catabolise their substrates more efficiently.

In aerobic situations hydrogen ions ( $H^+$ ) are combined with oxygen to form water. However, in anaerobic conditions hydrogen ions can be converted to molecular hydrogen by the hydrogenase enzyme which catalyses the reaction.



In gut anaerobes hydrogen ions are major electron acceptors. Most of the bacterial flora use this reaction (Gray and Gest 1965).

Rumen gas contains approximately 63 per cent carbon dioxide, 27 per cent methane, 7 per cent nitrogen with traces of hydrogen and hydrogen

sulphide and with small transient amounts of oxygen, (MacArthur and Miltimore 1961). The low hydrogen concentration is a result of the activity of methanogenic bacteria.

Many in vivo experiments with ruminants involve the use of rumen fistulas. Clapperton and Czerkawski (1969) used sheep with rumen fistulas to investigate the effect of time of feeding on methane production. Respiration chambers were used for gas collection. Maximal rates of methane formation occurred during and shortly after feeding when the concentration of dissolved hydrogen was maximal. When no food was given the rates of methane production continued to fall exponentially with a half life of about 24 hours. When long-chain unsaturated fatty acids were added to the sheep diet the amount of methane produced was significantly decreased (Czerkawski et al., 1966a). Infusion of saturated fatty acids into the diet also reduced the methane production (Czerkawski et al., 1966b).

Murray et al., (1976) estimated that  $11 \pm 2$  per cent of methane produced in the sheep hind gut was excreted via the anus and  $89 \pm 5$  per cent by the lungs. Of the methane produced in the rumen, 4 to 6 per cent was excreted by the lungs and the remainder by eructation. Using an isotope dilution technique and masks for collecting gas (Murray et al., 1978) measured the effect of feeding lucerne chaff to sheep. This method allowed gas production to be measured without surgical interference. Production of methane was found to be related to the energy intake. The technique used by Murray et al., (1976; 1978) enabled measurements to be made of the relative contributions to overall digestion of rumen and hind gut fermentation.

In vitro experiments have been performed using inoculations of ruminal contents in a fermentation apparatus. This type of technique

can provide information about rumen fermentation patterns. Pohland and Mancy (1969) investigated variables involved during anaerobic digestion of waste substances. Their results can be applied to ruminant model systems. The indices that were varied include gas production and composition, pH, concentration of volatile fatty acids and electrode potential (pE). Both pH and pE are particularly significant in analysing the anaerobic ecosystem since electron and proton transfer occur in an oxidation-reduction sequence.

Czerkawski and Breckenridge (1977) developed an artificial rumen, the Rumen Simulation Technique (Rusitec) to study, in a controlled manner, fermentation of various substrates. Both small-scale and large-scale apparatus were used by Czerkawski and Breckenridge (1969; 1972) to study the fermentation of various glycolytic intermediates and other compounds. They found that the amount of methane produced was dependent on the amount rather than the type of carbohydrate fermented. The average amount of methane formed was 6.0cals for every 100cals sugar fermented which was in good agreement with results obtained in vivo, (Czerkawski 1969).

Inhibitors of methane production, for example, carbon tetrachloride, trichloroethyl pivalate and trichloroethyl adipate were investigated in vitro (Czerkawski and Breckenridge 1975). Inhibition of methanogenesis led to an accumulation of hydrogen and increased rates of formation of reduced end-products, for example propionate. Czerkawski and Breckenridge (1979a, 1979b) investigated sequestration of bacteria and protozoa in the solid phase of the reaction mixture in Rusitec. Subsequently an inert solid matrix was provided for the micro-organisms to adhere to. This allowed a longer retention time of micro-organisms and enabled them to colonise sequentially which is closer to the in vivo situation.

The majority of work on methane production in animals has involved ruminants. However, methane production also occurs in the caeca of birds. Gasaway (1976) fed Rock Ptarmigan high and low cellulose diets in an attempt to use methane production as an index of caecal fermentation rates. He found great individual variation in methane excretion among ptarmigan. There were no differences in the production of methane in the two experimental groups. He concluded that the stoichiometry of methane formation in avian caeca may differ from that in ruminants. Methane excretion in rats and guinea-pigs was measured by Rodkey et al., (1972). They developed a closed circuit apparatus for measuring gases excreted in low concentrations. Using Spague-Dawley rats (mean body weight 293g) they showed no statistically significant difference between male and female rats with a mean and standard deviation for all rats of  $1.88 \pm 0.88 \text{ ml CH}_4/\text{kg.hr}$ . Methane production by germ-free rats was only  $0.51 \pm 0.34 \mu\text{l CH}_4/\text{kg.hr}$  and did not increase within 3 days after contamination of the animal with faeces from methane producing rats. This is consistent with the findings of Bond et al., (1971).

The human colon contains bacteria, undigested food residue with variable amounts of water and electrolytes, shed mucosal cells and other secreted and excreted substances. In the sigmoid colon and rectum there are about  $10^{11}$  bacteria per gram of wet weight. The small intestine contains fewer bacteria, about  $10^4$  per gram, (Drasar and Hill 1974). The bacterial flora is predominantly anaerobic with most species belonging to two genera, Bacteroides and Bifidobacterium. Human digestive enzymes have little if any effect on cellulose, hemicellulose, pectins and polyuronic acids. These materials make up a substantial fraction of the plant foods eaten by man, 10 to 20 per cent or more depending on diet. Bowel bacteria are therefore supplied with a variety of fermentable

substrates. About 20 per cent of a hemicellulose dose is degraded through passage in the gut, (Hill and Drasar 1975). Among the dominant bacteria of the large intestine, Bacteroides fragilis can utilise pectin, hemicellulose and starch (Bryant 1974). Ruminococcus bromii ferments starch to form ethanol and hydrogen.

Direct experimentation on lower gut fermentation of man has been limited largely to the analysis of faeces.

Most studies on human bowel microbiology have been on healthy men consuming a North American diet relatively high in animal protein and fat. In such people Bryant (1974) lists three bacterial species as being among the most important with respect to numbers. They are, Peptostreptococcus productus, Bacteroides fragilis including subspecies vulgatus, distosonis and thetaitaomicron and Ruminococcus bromii. These bacteria are all carbohydrate fermenters. Minor constituents of the bowel flora include Fusobacterium necrophorum, F. symboisum, Acidaminococcus fermentans and Veillonella species. Studies by Moore et al., (1978) show that faecal bacteria are similar in type and number to those of the colonic flora. In samples taken from various areas of the intestinal tract and the scrapings from the intestinal wall, the composition of the flora remained relatively constant from the ascending colon to the rectum. Moore et al., (1969) compared faecal flora of Americans, British, Indians, Japanese and Ugandans and found that generally a high carbohydrate diet decreased the numbers of Bacteroides species and increased the Enterococci and Eubacterium species. Short term dietary manipulations were not accompanied by significant changes in the numbers or species of bacteria in faeces (Drasar and Jenkins 1976). Bornside (1978) found that major groups of bacteria and their numbers per gram of faeces were not altered by diets either supplemented with or devoid of

dietary fibre. However, the total number of bacteria excreted daily is directly related to dietary fibre. Gorbach et al., (1967) also found that the numbers of bacteria recoverable from the same person were relatively stable and not significantly altered by simple variations in diet over 7 weeks to 7 months. It has been suggested (Finegold and Sutter 1978) that there may be changes in metabolic activity of intestinal bacteria in response to dietary manipulations.

Hydrogen and carbon dioxide are present in human colonic gas. Methane is not a characteristic component of the lower gut of man, (Levitt and Ingelfinger 1968). Methane content of flatus can vary from zero to 54 per cent, (Calloway 1968). The quantity and composition of human flatus has been investigated by Fries (1906); Kirk (1949); Levitt and Bond (1970). Using an argon washout technique Levitt and Bond (1970) found the composition of flatus to be extremely variable; nitrogen 23 to 80 per cent, oxygen 0.1 to 2.3 per cent, hydrogen 0.06 to 47 per cent, carbon dioxide 5.1 to 29 per cent and methane 0 to 26 per cent. In what is probably the earliest record of the composition of colonic gas, samples taken from newly executed prisoners were found to contain nitrogen, carbon dioxide and methane, (Magendie 1816).

Colonic gas and flatus samples can be collected by several methods. Hickey et al., (1972) attached a colostomy patch to the shaved buttocks. This method was preferred to an intrarectal tube and was equally efficient. In studies to determine the site and rate of hydrogen production in the intestine Levitt (1969a) used a multilumen tube to perfuse the intestine with nitrogen and a non-absorbable gas marker, sulphur hexafluoride ( $\text{SF}_6$ ). This constant infusion technique was also used by Levitt and Ingelfinger (1968).

Gases produced in the bowel as a result of bacterial activity are excreted in expired air. McIver et al., (1926) provided experimental

proof of diffusion and the conditions under which it proceeds in the gut. After injecting  $20\text{cm}^3$  of pure methane into a 25cm portion of dog intestine, which was then ligated and returned to the abdominal cavity, he found that approximately  $4\text{cm}^3$  were absorbed within one hour. The rate of absorption decreased with a reduction in the relative concentration of luminal gas. Hydrogen and methane concentrations are always higher in the lumen than in blood. The amount of each gas that diffuses will be proportional to its concentration in the gut lumen (Calloway 1968). According to gas laws the diffusion rate varies directly with surface area and inversely with membrane thickness. Removal of gas from the intestine is affected by the rate of blood flow in the gut and also the lungs. If the partial pressure is favourable 80 to 95 per cent of dissolved gases are usually cleared in a single passage through the lungs. Since the lungs are perfused several times the rate of gastrointestinal perfusion they should not limit gas removal. A gas that appears in expired air at a concentration of 100 parts per million (0.01 per cent) is removed at a rate approximating 40 to 50ml/hour (Calloway 1968). Decreased absorption from the intestinal lumen may be due to a reduced gut perfusion rate, mucosal thickening or impaired contact with gut membranes. Gut distension decreases the rate of gas removal. When intestinal motility is stimulated, the gas in the lumen will be expelled in flatus.

The proportion of gas excreted in the breath may vary and this has been the subject of several investigations. Levitt and Ingelfinger (1968) found an average of 14 per cent of hydrogen produced being excreted in the breath. This is probably a large underestimation resulting from the perfusion technique used. Calloway and Murphy (1968) state that hydrogen and methane are excreted mainly in the breath and

that this accurately reflects gas production. Normal production of intestinal methane would not be expected to exceed the capacity for intestinal absorption, although in situations where motility is stimulated excretion in flatus may occur. Even if some methane is passed as flatus there is ample evidence to show that the amount of gas excreted in the breath is proportional to the intestinal production. (Calloway 1968; Levitt 1969a; Levitt and Bond 1970). Levitt (1969a) compared breath hydrogen excretion with total hydrogen production and obtained correlations  $r = 0.94$  with infusion technique and  $r = 0.95$  with the washout technique.

Levitt (1969b) and Levitt and Levitt (1973) using a similar technique to McIver et al., (1926) investigated the intestinal absorption of gases as an indicator of the intestinal blood circulation. They injected known volumes of mixtures of helium, hydrogen and methane into isolated segments (stomach, small intestine and colon) of the rat. The rate of uptake of each gas into blood was determined by measuring the rate of pulmonary excretion into a closed system. The absorption data were fitted to a series of mathematical models of interaction between blood flow and diffusion. The authors state that if diffusion were the main factor limiting gas absorption, then the relative absorption rates would equal their relative diffusion rates. If the rate of blood flow was limiting, absorption would be defined by the relative solubilities in blood. They calculated the solubility of methane in blood to be  $0.032 \pm 0.0014$  (1SD) ml/ml blood at  $37^{\circ}$ . This is almost twice that of hydrogen ( $0.016 \pm 0.0016$  ml/ml blood). The approximate lipid-to-water solubility ratio for methane is 11 and for hydrogen is 3.4. The ratio of uptake of gases from the small intestine suggested nearly complete blood flow limitation. There was a degree of diffusion limitation from

the colon. Levitt and Levitt (1973) state that the rate at which a substance is absorbed by non-facilitated diffusion will be determined by 1) the rate of blood flow to the mucosa 2) the distance between the luminal contents and mucosal vessels and 3) the rate of diffusion of the substance through the unstirred layer and mucosa.

Reports of the proportion of subjects excreting methane in healthy populations range from 30 per cent (Calloway 1968; Levitt and Bond 1970; Bond et al., 1971) to 58 per cent (Pitt et al., 1980). Levitt and Ingelfinger (1968) reported that 50 per cent of their subjects excreted methane. A methane excreting subject was defined as excreting greater than or equal to one part per million above the atmospheric methane concentration. Calloway and Murphy (1968) found 60 per cent of healthy subjects excreting breath methane in concentrations greater than or equal to six parts per million. The reasons why a proportion of subjects excrete methane are uncertain and many factors which influence methane production and excretion have still to be elucidated.

One of the original papers by Bond et al., (1971) gives evidence of the influence of age, sex, familial relationships and the ingestion of lactulose on human methane excretion. They suggested dietary differences, differences in gut flora and genetic make-up as possible reasons for categorising subjects into two groups. Age, sex and the ingestion of lactulose had no effect on methane excretion although a probable familial component was identified. Methane excretion was not found to vary appreciably during the day or in response to food eaten (Calloway 1966). Tadesse and Eastwood (1978) and Tadesse et al., (1980) showed no effect of substrates on methane excretion measured in exhaled breath. There is a conflicting report by Pitt et al., (1980) showing increased methane excretion in some methane excreting subjects following

lactulose ingestion. The substrate for methane formation is something other than carbohydrate. Methane formation in the human colon appears to be unrelated to hydrogen formation (Calloway and Murphy 1968; Levitt and Ingelfinger 1968; Tadesse and Eastwood 1978; Tadesse et al., 1980). In vitro experiments using mixed bacterial cultures from the colon do not support this view. Carbohydrates have been shown to increase both hydrogen and methane production (Calloway et al., 1966).

Hydrogen and methane production are related in ruminants and in bacterial cultures (Hungate 1968; Wolfe 1971; Hungate 1976; Prins 1979). It seems that factors influencing methane formation in man are more complex and different from those in ruminants or in vitro. A number of factors may be acting together to bring about methane formation and a single factor such as diet or bacteria may not be the sole determinant.

In an effort to find a diagnostic use for breath methane measurements Levey and Balchum (1963) studied the prevalence of methane excretion in patients with constipation or faecal impaction, post-operative gastrointestinal patients, those with liver disease and those receiving intravenous alimentation. They found no differences in any of these clinical groups. The numbers of patients were very small and the influence of antibiotics on colonic bacteria was not considered. The only significant finding was that of Haines et al., (1977) who found 80 per cent of patients with large bowel carcinoma (N = 30) excreting methane compared to 40 per cent of their control patients without large bowel disease and 39 per cent of patients with non-malignant large bowel disease. The authors suggest that there may be a difference in anaerobic flora between these patient groups. This study does not indicate whether carcinoma of the large bowel is a consequence of methane excretion or whether methane excretion is secondary to carcinoma. A prospective study is suggested. More recently there has

been a finding of 30 per cent of patients with unresected large bowel carcinoma (N = 20) excreting breath methane (Karlin et al., 1980a).

To summarise, various methods for studying methane production include 1) in vitro culture techniques with pure and mixed bacterial species 2) small and large scale fermenters and the artificial rumen simulation technique (Rusitec) 3) analysis of ruminant gases excreted in flatus, breath and during eructation 4) analysis of human flatus and expired breath.

The work in this thesis mainly involves detection of methane in human breath. Attempts have been made to isolate factors which may influence methane production. Supportive evidence has also been obtained from work with pure methanogenic bacteria and other intestinal bacteria. An animal model, the rat, has also been investigated. The general questions posed are, "Why do all human subjects not excrete methane?" and "What factors influence the excretion of methane?". Diets, faecal components and bowel function have been investigated in methane excreting and non-methane excreting subjects in populations with different methane prevalences. Healthy subjects and patients with clinically defined conditions have also been studied.

CHAPTER 2  
SUBJECTS AND METHODS

## 1. PARTICIPANTS - HUMANS

Human subjects in this study were volunteers living in Edinburgh aged 16 to 79 years. Many were members of staff and students (35 men, 25 women) in the hospital and the remainder lived in the Muirhouse and Pilton area of North Edinburgh (39 men, 43 women, including 34 married couples). These subjects were selected from a group of patients considered by their family doctor to be free of gastrointestinal disease and who were willing to co-operate in this study. All subjects were in good health at the time of participation and were not taking antibiotics or laxatives.

The clinical patients investigated either

- a) attended the Gastrointestinal Unit, Western General Hospital, (117 men, 147 women aged from 16 to 73 years, mean age 49 years).
- b) were patients in wards in the Western General Hospital, for example, Urology, Orthopaedics, Gynaecology (35 men, 29 women aged from 17 to 86 years, mean age 48 years).
- c) were in Geriatric wards of the City and Royal Victoria Hospitals (17 men, 49 women aged from 67 to 98 years, mean age 79 years).
- d) attended the Peripheral Vascular Clinic, Royal Infirmary of Edinburgh (44 men, 16 women aged from 45 to 77 years, mean age 62 years). Further clinical details of these subjects are given in Chapter 5.

## ANIMALS

The rats used were male and female Wistars of varying ages obtained from a colony of inbred rats maintained in the Animal Unit

Teaching and Research Centre, Western General Hospital. These animals were kept on a 12 hour light/dark cycle with constant recirculation of filtered air (5 to 6 times/hour) and the temperature was maintained at 20 to 22<sup>0</sup>C.

## 2. GAS SAMPLING TECHNIQUES - HUMAN

The breath sampling method for end-expiratory air used in this study is the modified method of Haldane and Priestley (1905), used by Metz et al., (1976a). A 9cm glass mouth-piece is attached to a 150cm long polypropylene tube with a 12mm internal diameter. 18cm from the proximal end of the tube is a hole into which is inserted a three-way tap for connecting a 50ml syringe (polypropylene) for sampling air from the lumen of the tube. The subject whose breath is to be sampled sits in a chair and blows into the tube through a mouth-piece with one forced expiration following a normal inspiration. At the end of the forced expiration the mouth-piece is sealed with the tongue and a 50ml sample of air is withdrawn from the tube.

## ANIMALS

The method used in this study for animal gas collection is the modified method of Gumbmann and Williams (1971). Production of methane and hydrogen is estimated from the change in gas composition of the closed chamber containing a single animal for a period of 15 minutes. The animal chamber is a perspex rectangular box (30 x 20 x 20cm, capacity 12l) with all joints recessed. The lid has four holes into

which are inserted three-way taps and there is a groove with a rubber seal around the lid to ensure a tight fit. There are also two handles with springs to keep the lid firmly down. Air is circulated at a constant speed from the chamber by a diaphragm type air pump (PXW-520-S, A Gallenkamp and Co. Ltd) with an output of 3.25 l/min and is passed through soda lime and silica gel for the absorption of carbon dioxide and excess water vapour. A manometric oxygen valve is connected in series which allows an excess of oxygen to flow continuously through the left arm of the U-tube valve. Consumption of oxygen by the animal produces a slight negative pressure causing oxygen to bubble through the light weight oil in the U-tube and enter the otherwise closed system. In practise it was found very difficult to regulate the oxygen flow from the cylinder to compensate for the animal's utilisation of oxygen and so the method was further modified.

The animal was placed in the box which was then sealed and left for 15 minutes. A 50ml gas sample was then withdrawn. The chamber was cleaned after each animal tested. The large capacity of the box allowed the animal to breath normally for 15 minutes without the addition of oxygen. However, this raised the lower detection level of methane and hydrogen. Based on the sensitivity of the katharometer detector and the volume of the chamber the lower limit of detection was approximately  $0.09\mu\text{mol/l}$  which requires a production rate of about 0.094 ml/hr of methane and hydrogen in a 200g rat. The excretion rate was calculated from the concentration of methane in the chamber (12000ml) minus the volume of the rat (density assumed to be 1.00). Similar modified methods have been used by Rodkey et al., (1972), Levitt and Levitt (1973), Bond et al., (1978) with rats and by Gasaway (1976) with the Rock Ptarmigan Lagopus Mutus.

The ability of the chamber to retain methane and hydrogen without loss was verified by flushing the chamber with a 4.5 $\mu$ mol/l mixture of methane and hydrogen for 15 minutes until full and then sealing the lid. There was no loss of either methane or hydrogen over 105 minutes. The mean percentage loss after 24 hours over 5 trials was 1.5% methane and 5.4% hydrogen.

### 3. METHOD OF GAS ANALYSIS

In the gas chromatographic detection of methane and hydrogen two different types of detectors are in general use i.e. Flame Ionization Detector for methane and Katharometer Detector for hydrogen. This is reputed to be due to differences in sensitivity of the two systems for the two gases. The method used in this project was that of Tadesse et al., (1979). The analytical instruments used were a gas-solid chromatograph (GSC) (Pye Unicam, Series 104) with a Katharometer Detector (KD) and a 1mv pen recorder for display of values (Philips PM 8000). The chromatographic working conditions e.g. column packing, detector temperature, carrier gas and flow rate are shown in Table 2(a). With this method the first peak is hydrogen with a retention time of 65s, the second peak oxygen with a retention time of 103s and the third is methane with a retention time of 300s. Peak resolution between hydrogen and oxygen is 3.3 and that between oxygen and methane is 6.0. Figure 2(a). Varying attenuations were used for the gas analysis depending on the source of the gas sample and the expected range of concentrations in the test sample, e.g. 2 attenuation for breath samples, 1000 and 50 for gas samples from

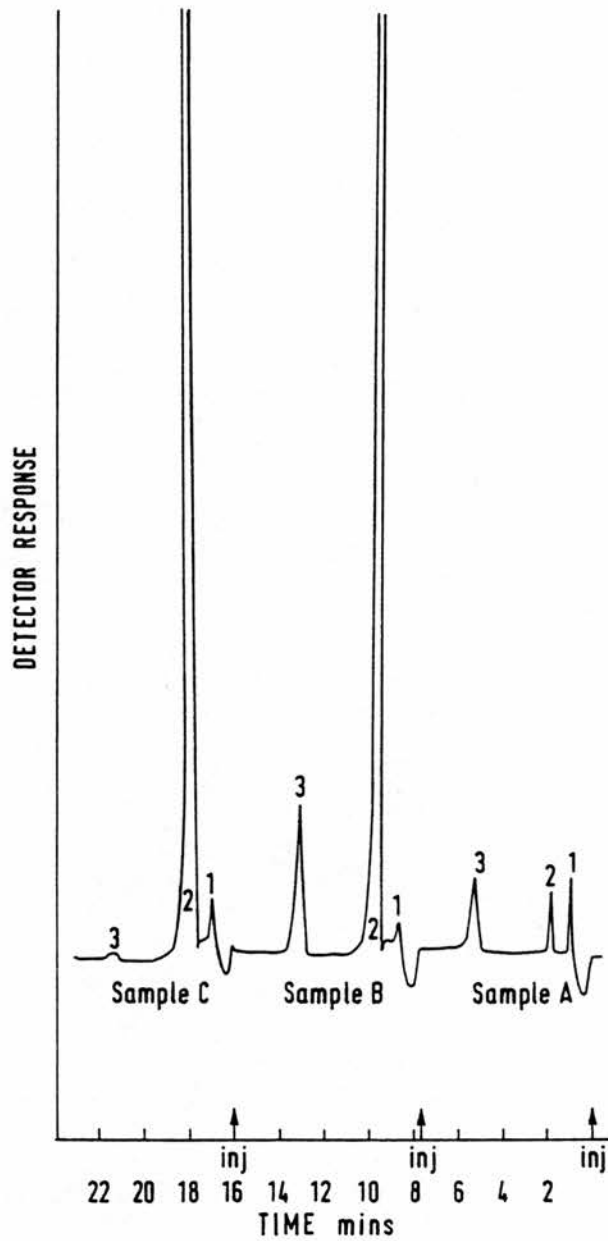


Figure 2(a) Chromatogram of breath and standard gas samples using a katharometer detector.

Sample A 0.9  $\mu\text{mol/l}$  standard gas sample  
 B breath sample from a methane producer  
 C breath sample from a non-producer

Peak 1 Hydrogen  
 2 Oxygen  
 3 Methane

See Table 2(a) for chromatographic working conditions

bacterial culture bottles. Calibration curves were constructed using serial dilutions of pure methane (Air Products Ltd.) and hydrogen (BOC Ltd.).

This method accurately detected methane concentrations of  $0.09\mu\text{mol/l}$  and the coefficient of variation was 4% for repeat analysis ( $n = 20$ ) of 0.9 and  $4.5\mu\text{mol/l}$  hydrogen and methane standards (Rank Hilger, Kent, Great Britain).

A comparison of the above method with the detection of methane using a Flame Ionization Detector was made. Starting with the manufacturer's suggested working conditions for the chromatograph (Pye Unicam, Series 104) each of the following variables were independently varied e.g. column temperature, packing and length, carrier gas flow rate, injection loop size and attenuation whilst keeping the others constant and the effect on the sensitivity, peak resolution and retention times were assessed. Excess carbon dioxide and water vapour were absorbed by soda lime and silica gel prior to injection. These studies enabled suitable working conditions, within the limits of the gas chromatograph to be chosen (Table 2(a)). Gas samples of 100% carbon dioxide and 95% oxygen, 5% carbon dioxide were analysed to aid the identification of the first peak. With the Flame Ionization Detector the first peak was oxygen with a retention time of 42s and the second peak was methane with a retention time of 78s. Figure 2(b) Peak resolution was 12. The relationship between different concentrations of standard gases e.g. 4.5, 2.25, 0.9 and  $0.45\mu\text{mol/l}$  and the deflection on the recorder (mv) was linear. The coefficient of variation for repeat analysis ( $N = 20$ ) of 0.9 and  $4.5\mu\text{mol/l}$  methane standards was 4%.

#### 4. DIET ANALYSIS - HUMANS

Diet data was collected from volunteers by a dietician using a

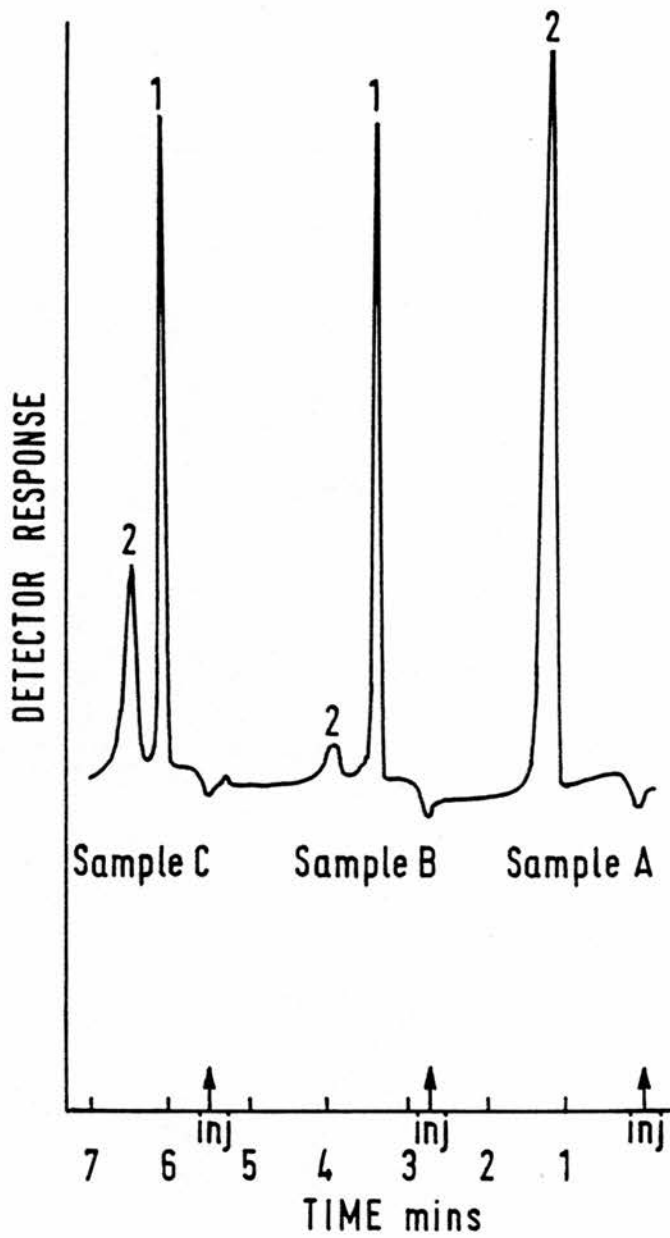


Figure 2(b)

Chromatogram of rat, room air and standard gas samples using a flame ionization detector.

Sample A 0.9 $\mu$ mol/l standard gas sample  
 B room air sample  
 C gas sample from a rat

Peak 1 Oxygen  
 2 Methane

See Table 2(a) for chromatographic working conditions

24 hour recall diet history and a diet diary kept over one week. The dietician made daily visits and conducted an interview. The daily dietary intakes were calculated from the food tables of McCance and Widdowson (1960) and the guide to calculating intakes of dietary fibre by Southgate et al., (1976). A computer programme based on this information was used (McCance and Widdowson's computer tape M.A.F.F. Publications).

### ANIMALS

During the animal experiments several dietary regimes were used and these are briefly described below.

- a) Spratt's Laboratory Small Animal Diet (Autoclaved). Table 2(b)
- b) 50% (500g/kg) bran plus Spratt's. Table 2(c)
- c) Flexical elemental diet. Table 2(d)
- d) 20% (200g/kg) gum acacia plus Flexical
- e) 20% (200g/kg) gum acacia plus Oxoid Breeding diet. Table 2(e)
- f) Minced meat (500g/kg) and egg (500g/kg) diet

All diets were fed ad libitum and the daily intakes (g) were measured. Water was unrestricted.

Further details about gum acacia and the minced meat and egg diet are in Chapter 6.

### 5. FAECAL COMPONENT ANALYSIS

7 day stool collections were made by volunteers. Individual stools within each collection period were weighed and pooled for each

subject and homogenised mechanically using a Colworth Stomacher, model 3500. An aliquot of known weight was freeze dried. The resultant powder was weighed to determine the dry weight of the stools. An aliquot of the powder (50mg) was used for bile acid analysis (Evrard and Janssen 1968). Faecal fat was measured by titration of extracted fatty acids as described by Varley (1967). Faecal electrolytes were measured by flame photometry and atomic absorption spectrophotometry after charring with nitric acid.

Volatile fatty acid (VFA) analysis was performed on rat faeces and on caecal contents. The method was based on that of Spiller et al., (1980).

Four standard solutions were made up

1. 10 $\mu$ l stock standard<sup>\*</sup> + 0.79ml distilled water + 50 $\mu$ l internal standard<sup>\*\*</sup> + 100 $\mu$ l orthophosphoric acid.
2. 25 $\mu$ l stock standard + 0.775ml distilled water + 50 $\mu$ l internal standard + 100 $\mu$ l orthophosphoric acid.
3. 50 $\mu$ l stock standard + 0.75ml distilled water + 50 $\mu$ l internal standard + 100 $\mu$ l orthophosphoric acid.
4. 100 $\mu$ l stock standard + 0.7ml distilled water + 50 $\mu$ l internal standard + 100 $\mu$ l orthophosphoric acid.

\* Volatile fatty acid stock standard contained 1g each acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid per 100ml water at pH 7.0.

\*\* Internal stock standard contained 1 g  $\beta$  methylvaleric acid per 100ml water at pH 7.0.

Each animal's faecal collection was weighed, pooled and freeze dried. Approximately 100mg of the resultant powder was used for the test solution along with 0.8ml distilled water, 50 $\mu$ l internal standard and 100 $\mu$ l orthophosphoric acid. All solutions were mixed thoroughly,

the test solutions requiring centrifugation at 3,000rpm for 10 minutes to separate the ether layers. The solutions were extracted with 3ml redistilled diethyl ether three times and the extracts were combined and mixed. The volatile fatty acids were separated using a Flame Ionization Detector gas chromatograph, temperature programmed (80°C to 150°C at 16°C/min) with column packing SP2250. The flow rate of the carrier gas (oxygen free nitrogen) was 40ml/min. Figure 2(c). Calibration curves were constructed for each volatile fatty acid, plotting the ratio of volatile fatty acid to internal standard peak heights against volatile fatty acid concentration. Standard solutions were made up and analysed and calibration curves drawn on each day of analysis.

## 6. INTESTINAL TRANSIT TIME AND CAECAL RETENTION TIME MEASUREMENTS

Two methods of assessing gastrointestinal transit time were used.

1. Measurements of intestinal transit time were made using barium impregnated markers (S.G. 1.1) as described by Hinton et al., (1969). Markers in the frozen stool were made visible under a fluoroscan and counted. The time when 80% of the markers were passed was taken as the transit time. These markers also gave an indication of the completion of collection.
2. Measurement of whole gut transit time with barium impregnated markers using single stool analysis (Cummings and Wiggins 1976). Three different shapes i.e. cubes, circles and cylinders of radio-opaque polyethelene pellets of approximately the same specific gravity and similar behaviour in the gut were

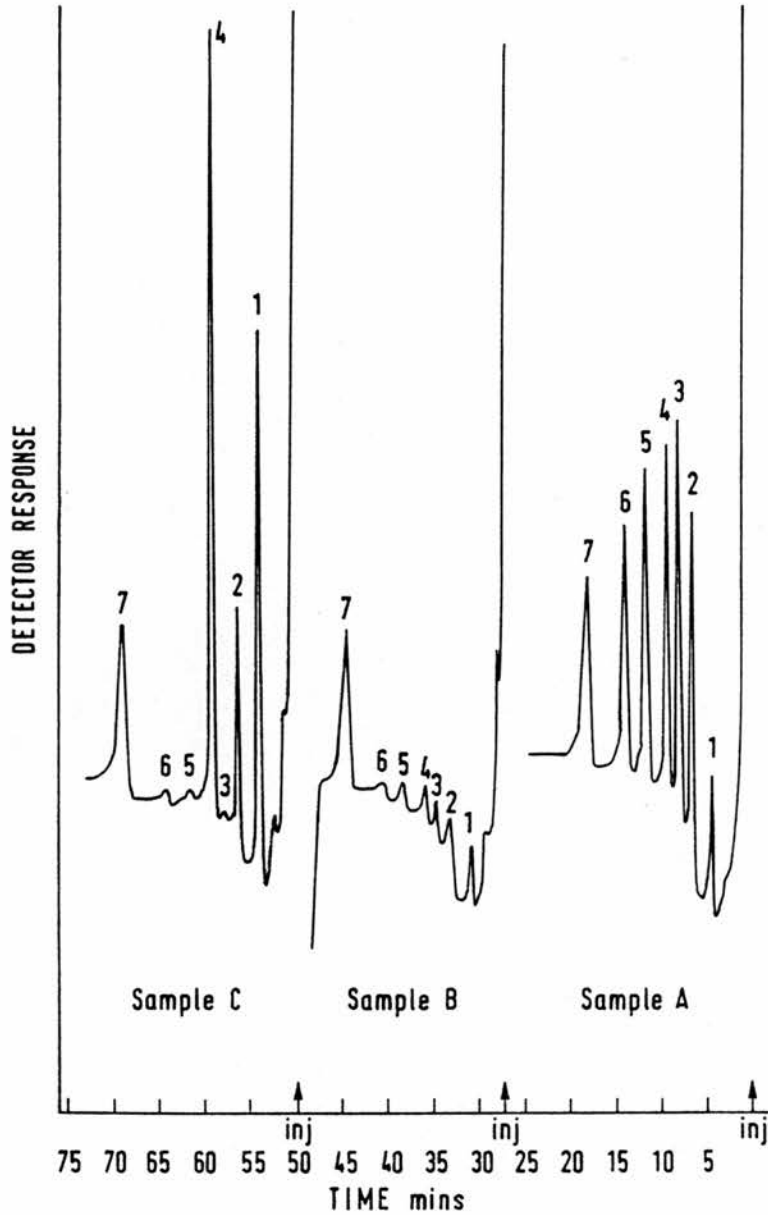


Figure 2(c) Chromatogram of volatile fatty acids using a flame ionization detector.

Sample A 50 $\mu$ l standard sample  
 B rat faecal sample  
 C rat caecal sample

Peak	1	acetate	4	butyrate
	2	propionate	5	isovalerate
	3	isobutyrate	6	valerate
			7	methyl valerate

used. Twenty of the specified shape of marker for the particular day were administered with breakfast on three successive days and the first stool passed on the fourth day collected. The time of administration of each marker and the collection of the stool was noted. After freezing, the stool was X-rayed and the different markers counted. The transit time was calculated using the formula

$$SST = \frac{t_1 s_1 + t_2 s_2}{s_1 + s_2}$$

where SST is a single stool transit time,  $t_1$  and  $t_2$  the time from ingestion of markers to time of stool collection of the two marker/shapes found in greatest number in the stool and  $s_1$  and  $s_2$  the number of each marker in the stool. This method was also used as an indication of residence time in the large intestine. Plain abdominal X-rays were taken on days 2, 3 and 4 and the number of different markers were counted in the various areas of the large intestine e.g. caecum/ascending colon, transverse colon and descending colon/rectum.

## 7. HISTOLOGY

Gut and liver tissue was obtained from rats for histology. Pieces of jejunum, ileo-caecal region and colon were removed and opened. Faeces were removed from the villous surface by flushing with saline and the mesenteric surface was flattened and attached to a piece of white card. 3mm thick transverse sections through the largest liver

lobe were also attached to white card. The tissues were then fixed in 10% buffered Formalin (pH 7.0). Subsequent sectioning and staining (Haematoxylin and eosin) was performed in the Research Pathology Laboratory, Western General Hospital.

## 8. STATISTICS

Several statistical methods were used in the following studies including Student's 't' test,  $\chi^2$  test, and the non-parametric Wilcoxon Signed Rank Test (Maxwell, 1970; Documenta Giegy 1973).

Linear regression analysis was performed using the Texas calculator model T1-51-III.

Logistic regression analysis using the Edinburgh Multi-access System (EMAS) computer was performed by Mr. W. Adams, Medical Computing and Statistics Unit.

TABLE 2(a)

CHROMATOGRAPHIC WORKING CONDITIONS

	<u>KATHAROMETER DETECTOR</u>	<u>FLAME IONIZATION DETECTOR</u>
Column type	Glass	Glass
size	2.6m (4 mmid)	1.5m (4 mmid)
Mesh type	M.S. 5A	M.S. 5A
size	60/85	60/85
Column temp.	50°	125°
Detector temp.	100°	
Carrier gas	Oxygen free nitrogen	Oxygen free nitrogen
Flow rate	65ml/min	40ml/min
Sampling type	loop 10ml	loop 10ml
temp.	Room temp.	Room temp.
Attenuation	2	1 x 10 <sup>2</sup>
Display	Pen recorder	Pen recorder
Chart speed	5mm/min	1cm/min

TABLE 2(b)

SPRATT'S LABORATORY SMALL ANIMAL DIET (AUTOCLAVED)CRUDE COMPOSITION

8.6%	moisture
21.3%	crude protein
3.4%	crude fat
5.6%	mineral matter
2.2%	crude fibre
48.0%	calculated digestible carbohydrates
4.154	gross energy Kcal/g diet
3.425	digestible energy Kcal/g diet (calculated)
41.8%	starch
2.8%	sugars (sucrose)

Information provided by Spratt's

TABLE 2(c)

COMPONENTS OF WHEAT BRAN

Crude fibre	8.9%
Protein	14.3%
Fat	0.2%
Carbohydrate (starch)	17.4%

TABLE 2(d)

FLEXICAL COMPLETE ELEMENTAL DIET

Each 100g powder contains as a minimum:

Protein	g	9.2	
Carbohydrate	g	66.9	(monosaccharides)
Fat	g	14.2	
Metabolisable energy	MJ	1.85	(440 Kcal)

TABLE 2(e)

RAT BISCUIT DIET - OXOID BREEDING DIET

Ingredient	%
Maize	10.0
Wheat	40.0
Maize Germ	7.5
Soya Extract	27.5
Fish Meal	5.0
Molasses	2.5
Fat	1.25
Distillers Dried Solubles	2.5

Supplements, Amino acids, Vitamins and Minerals to 100%

Crude proteins	%	22.9
Oil	%	4.0
Fibre	%	3.56

CHAPTER 3

THE PREVALENCE OF BREATH METHANE EXCRETION IN NORMAL POPULATIONS

It has been shown in other studies that the proportion of adults excreting methane in the breath can vary from 33 per cent (Bond et al., 1971) to 58 per cent (Pitt et al., 1980). Methane producers are defined as excreting more than  $0.045\mu\text{mol/l}$  (1ppm) above the methane concentration in air, which is usually  $0.09\mu\text{mol/l}$ . The remainder are defined as non-producing subjects if they excrete less than or equal to  $0.045\mu\text{mol/l}$  (1ppm). (Bond et al., 1971). These workers showed that methane excretion is not related to age or sex although there may be a familial trait. Methane excretion was found not to vary in response to the type of food eaten (Calloway 1966) and was not affected by fasting for periods up to 24 hours. (Levitt and Ingelfinger 1968; Tadesse et al., 1980).

Bond et al., (1971) also demonstrated that the breath methane concentration is strongly correlated ( $p < 0.001$ ) with the respiratory excretion rate.

In the following series of investigations the incidence of methane production in two normal, but distinct, population groups living and working in the North of Edinburgh was studied.

The relationship between the methane concentration in end-expiratory breath and the rate of pulmonary excretion was confirmed. The hourly and daily fluctuations in breath methane excretion were studied whilst on a normal diet. The diets, faecal characteristics and bowel function of methane producers and non-producers were compared between and within the two population groups.

METHODS

Two population groups were studied. The first group consisted of hospital staff and students; 35 men and 25 women aged from 20 to 62 years (mean 29 years).

1. All subjects provided two random breath samples using the method previously described, whilst taking their habitual diets.
2. 26 of these subjects who worked in or near the laboratory provided breath samples every hour from 09.00 to 17.00 hours whilst taking their normal diets e.g. breakfast around 07.30hrs, lunch at 12.30hrs and a cup of coffee at 15.30hrs.
3. 10 subjects in the laboratory provided non-fasting breath samples at the same time each weekday morning for up to 8 consecutive weeks.
4. The rate of pulmonary methane excretion was measured in 3 methane producing subjects. Breath was collected by means of a Douglas bag over 3 minutes whilst resting after an initial adaptation period. The number of breaths were counted and the total gas volume was measured by a gas meter (Tissot), corrected to body temperature and pressure, saturated (BTPS) and the volume/minute calculated. Methane concentration was measured in the mixed expiratory air from the bag and in end-expiratory breath samples taken before and after the respiratory test.
5. 25 subjects (21 men, 4 women, age 29 to 46 years, mean 38 years) also provided diet data. 15 of the male subjects collected stools for 5 days. These 15 subjects worked in or close to the laboratory and participated in nutrition experiments.

The information on dietary intakes and faecal components was obtained during control periods prior to an experiment. The remaining 10 subjects were university students who only provided diet data.

The second group of subjects consisted of 82 healthy volunteers, 39 men and 43 women aged from 16 to 79 years (mean 42 years) living in the Muirhouse and Pilton area of North Edinburgh. They were mainly married couples. (34 couples) including 4 families (parents and children) recruited by their family doctor.

1. All subjects provided two random breath samples. Air samples were also collected from the rooms in which the subjects were tested.
2. 56 subjects participating in a bowel function study, 26 men and 30 women aged from 16 to 79 years (mean 58 years) provided diet data. This was obtained by the methods previously described. The dietician visited the subjects every day whilst they were keeping a diet diary.
3. These 56 subjects made 5 day stool collections. Measurements of intestinal transit time were made using barium impregnated markers and the stools were analysed for faecal bile acids, fat, electrolytes and neutral sterols.
4. 15 male subjects aged from 20 to 61 years (mean 37 years) who were working in a bakery in the North west of Edinburgh were recruited for a nutrition experiment and provided two breath samples. Their diet histories were assessed by a dietician. 5 day stool collections were also made.

Breath methane concentrations were taken as the difference between the mean of the two samples and the room air sample. Methane

producers were defined as those subjects excreting methane in concentrations greater than or equal to  $0.09\mu\text{mol/l}$  above room air concentrations. No breath samples were taken within 10 minutes of a subject smoking.

### ANALYSIS OF RESULTS

In these investigations several statistical methods have been used. Student's "t" test was used to compare results from methane producers and non-producers. Linear regression analysis was used to associate the concentration of methane excreted with dietary intakes and faecal components. The numbers of methane excreters in each group were compared using  $\chi^2$  test.

Logistic regression analysis was performed by Mr. W. Adams of the Medical Computing Unit using the Edinburgh Multi-access System (EMAS).

This statistical method related the characteristic of methane production with

- (a) faecal components and bowel function e.g. stool wet and dry weight, transit time, faecal fat, bile acids, neutral sterols, electrolytes.
- (b) dietary intakes expressed as the food categories of McCance and Widdowson (1960) e.g. beef, other meats, offal, fish, dairy produce, fruit and vegetables, processed foods, cereals, sugar and preserves, alcohol. The results from group 2 subjects were analysed with this technique.

## RESULTS

Table 3(a) shows the number and percentages of methane producers in each sex for the hospital staff and student subjects (group 1). The concentration of methane excreted ranged from 0.27 to 2.43 $\mu\text{mol/l}$  above room air concentrations and was not influenced by age ( $r = 0.13$ , N.S.). There were no significant differences between the proportion of male and female methane excreters, (34% and 32% respectively).

The number and percentage of methane producers in each sex for the local volunteer subjects (group 2) are shown in Table 3(b). The concentration of methane ranged from 0.09 to 2.1 $\mu\text{mol/l}$  above room air concentrations. Methane excretion was not influenced by age ( $r = 0.067$ , N.S.). Of the 34 pairs of spouses studied, 13 pairs were discordant for methane excretion i.e. only one member excreted methane and 21 pairs were concordant with both members excreting ( $N = 15$ ) or not excreting methane ( $N = 6$ ). In each of the 4 families studied both parents and all their children excreted methane. There were no significant differences between the proportion of male and female methane excreters. (80% and 60% respectively). Figure 3(a) illustrates the range of methane excreted. 9 of the bakery subjects excreted methane which ranged from 0.13 to 1.52 $\mu\text{mol/l}$  above room air concentrations.

### HOURLY AND DAILY VARIATIONS

26 subjects from group 1 provided breath samples every hour. 13 of these subjects produced methane which varied minimally. Table 3(c) shows the mean, standard deviation (SD) and coefficient of variation (CV) of methane concentration ( $\mu\text{mol/l}$ ) for these 13 subjects. Methane was not detected in the breath of the remaining subjects. The mean, SD, and CV of methane concentrations in 6 methane producing subjects over

TABLE 3(a)  
NUMBER OF METHANE PRODUCERS,  
MEAN AND STANDARD DEVIATION METHANE CONCENTRATION FOR GROUP 1  
(HOSPITAL STAFF AND STUDENTS)

	<u>Men</u>	<u>Women</u>
Number of subjects	35	25
Number of methane producers	12 (34%)	8 (32%)*
Mean $\pm$ SD methane concentration ( $\mu\text{mol/l}$ )	$0.77 \pm 0.54$	

\* men compared to women N.S. (not significant) using  $\chi^2$  test

TABLE 3(b)  
NUMBER OF METHANE PRODUCERS,  
MEAN AND STANDARD DEVIATION METHANE CONCENTRATION FOR GROUP 2  
(LOCAL VOLUNTEERS)

	<u>Men</u>	<u>Women</u>
Number of subjects	39	43
Number of methane producers	31 (80%)	26 (60%)*
Mean $\pm$ SD methane concentration ( $\mu\text{mol/l}$ )	$0.77 \pm 0.50$	

\* men compared to women N.S. (not significant) using  $\chi^2$  test

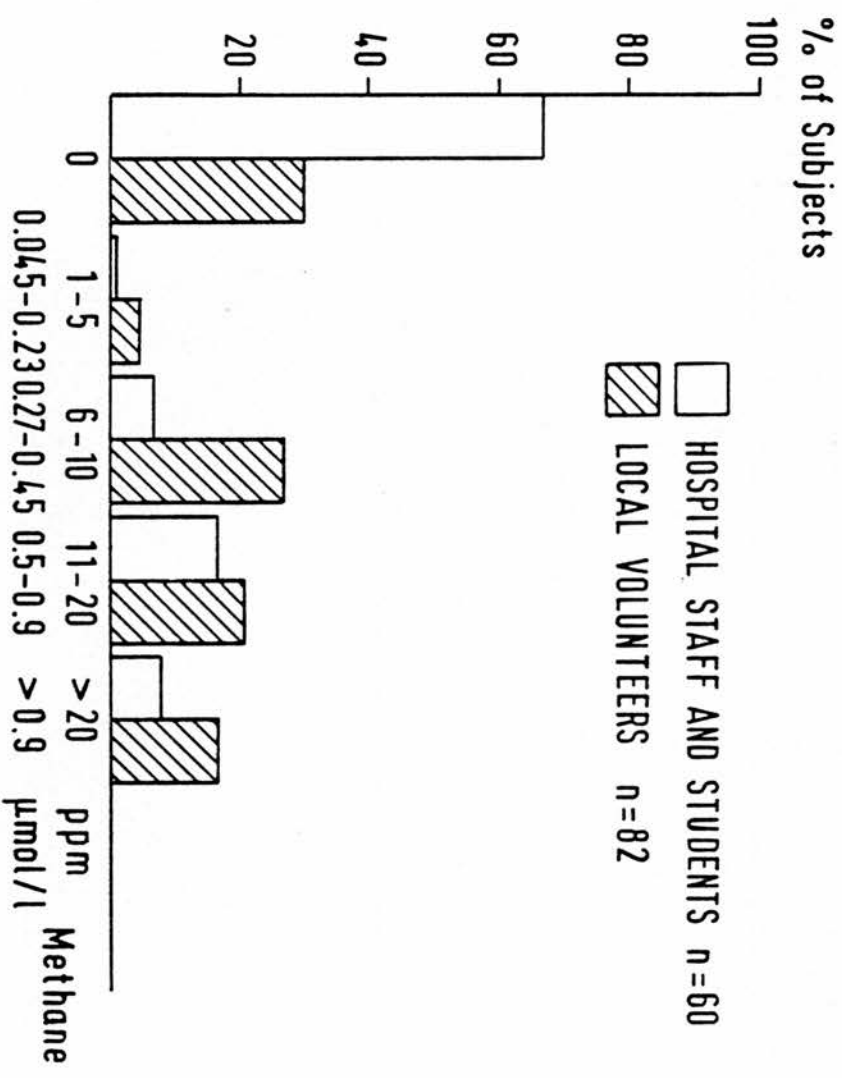


Figure 3(a) Breath methane concentrations ( $\mu\text{mol/l}$ ) in healthy subjects

TABLE 3(c)

<u>Subject</u>	Hourly methane concentration ( $\mu\text{mol/l}$ ) over 8 hours			Daily methane concentration ( $\mu\text{mol/l}$ ) over 2 to 8 consecutive weeks		
	<u>Mean</u>	<u>SD</u>	<u>CV</u>	<u>Mean</u>	<u>SD</u>	<u>CV</u>
1	1.13	0.24	21.2	0.88	0.47	53.4
2	1.24	0.32	25.8	1.21	0.54	44.6
3	2.40	0.45	18.7	1.48	0.58	39.1
4	0.22	0.05	22.7	0.27	0.16	59.2
5	0.96	0.20	20.8	0.45	0.26	57.7
6	1.24	0.38	30.6	1.01	0.67	66.3
7	0.61	0.19	31.1			
8	0.75	0.13	17.3			
9	0.63	0.14	22.2			
10	1.15	0.19	16.5			
11	0.63	0.21	33.3			
12	0.83	0.13	15.6			
13	0.56	0.13	23.2			

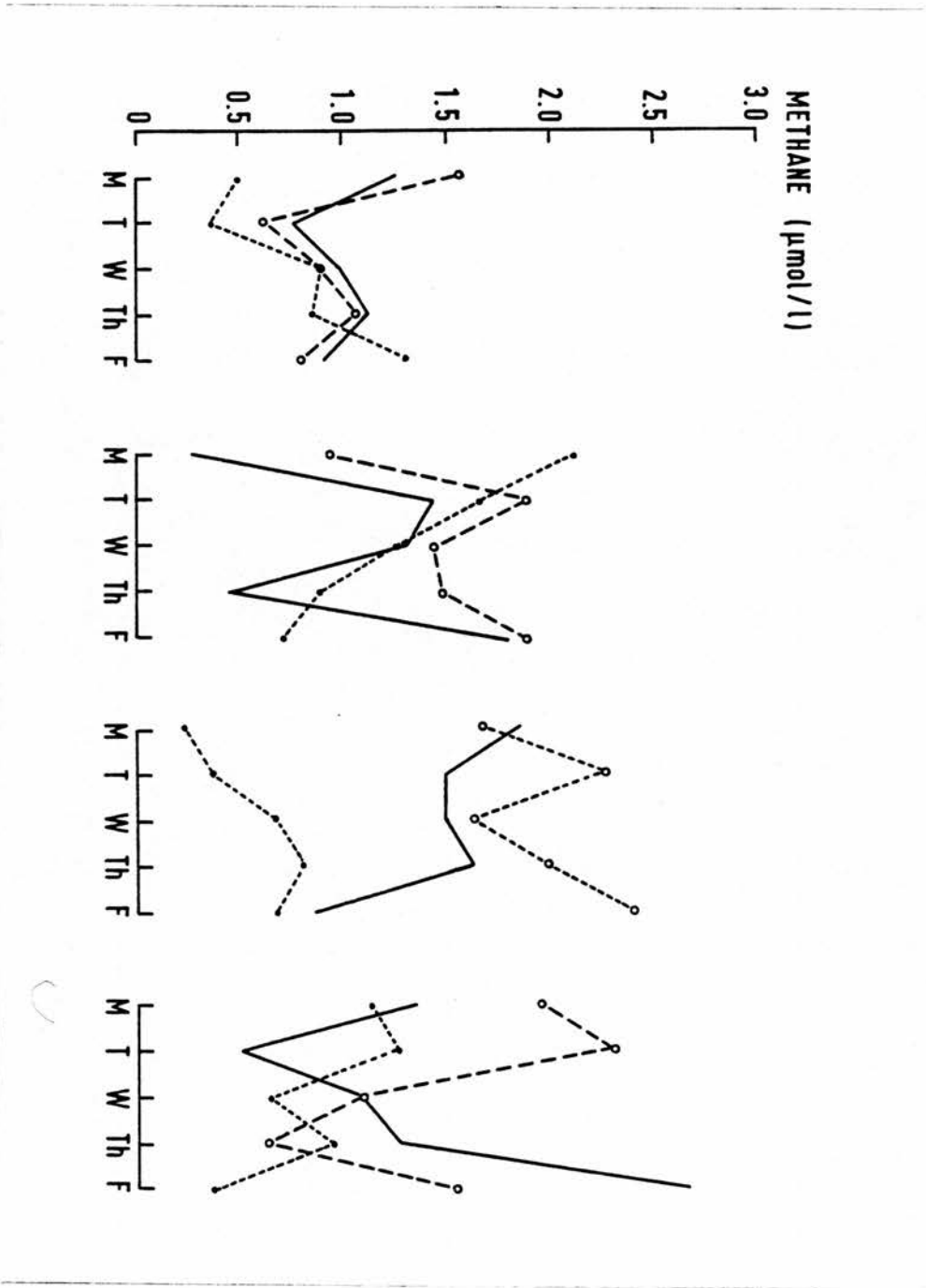


Figure 3(b) Daily variations in methane concentration ( $\mu\text{mol/l}$ ) over 4 weeks in 3 subjects

2 to 8 consecutive weeks are also shown in Table 3(c). Figure 3(b) illustrates the daily methane concentrations in 3 subjects over 4 consecutive weeks.

#### PULMONARY METHANE EXCRETION RATE

The results of the pulmonary methane excretion rate measurements are shown in Table 3(d).

End-expiratory breath methane concentrations were reduced after the subjects had breathed into the Douglas bag for 3 minutes. Concentrations for the 3 subjects before the test were 2.11, 2.07 and  $0.77\mu\text{mol/l}$  and after the test 1.49, 1.58 and  $0.59\mu\text{mol/l}$ .

#### DIETARY INTAKES

Tables 3(e)(f) contain the daily dietary intakes for group 1 and group 2 subjects respectively. Daily dietary intakes of methane producers and non-producers are compared in Tables 3(g)(h). There were no significant differences between the dietary intakes of methane producers and non-producers in either group. Within the methane excreting subjects ( $N = 34$ ) in group 2 there was an association between the concentration of methane excreted and the intake of the pentose fraction of non-cellulosic polysaccharide ( $r = 0.44$ ,  $p < 0.01$ ) (Figure 3(c)) and lignin ( $r = 0.40$ ,  $p < 0.05$ ). The intakes of pentose and lignin were correlated ( $r = 0.82$ ,  $p < 0.001$ ). Methane excretion was not associated with the intake of any other food category. There were no associations between the concentration of methane excreted and dietary intakes in the group 1 subjects. Dietary data for the 15 male nutrition experiment subjects is shown in Table 3(i). Since

TABLE 3(d)

RELATION BETWEEN BREATH METHANE CONCENTRATION ( $\mu\text{mol/l}$ ) AND RESPIRATORY EXCRETION RATE ( $\mu\text{mol/min}$ )

Subject	End expiratory ( $\text{CH}_4$ )* $\mu\text{mol/l}$	Mixed expiratory ( $\text{CH}_4$ ) $\mu\text{mol/l}$	Respiratory excretion rate $\mu\text{mol/min}$
1	1.83	1.53	10.40
2	1.80	1.22	7.16
3	0.68	0.54	4.00

\*Mean of end-expiratory samples before and after the test

TABLE 3(e)

DAILY DIETARY INTAKES FOR GROUP 1 SUBJECTS (N = 25)

<u>Food category (g)</u>	<u>Mean</u>	<u>1SD</u>
Protein	84	24
Fat	117	40
Carbohydrate	268	108
Energy MJ	10.6	3.5
Saturated fat	105	41
Unsaturated fat	9.4	6.9
Cholesterol	0.4	0.2
Dietary fibre	17.8	10.5
Non-cellulosic polysaccharide	13.8	7.7
Hexose	6.6	2.9
Pentose	4.5	3.7
Uronic acid	2.8	1.6
Cellulose	4.4	2.1
Lignin	1.6	1.4

Dietary intakes were calculated using the food tables of McCance and Widdowson (1960) and Southgate et al., (1976)

TABLE 3(f)

DAILY DIETARY INTAKES FOR GROUP 2 SUBJECTS (N = 56)

<u>Food category (g)</u>	<u>Mean</u>	<u>1SD</u>
Protein	68	19
Fat	99	32
Carbohydrate	226	83
Energy MJ	8.9	2.9
Saturated fat	93	32
Unsaturated fat	6.1	4.5
Cholesterol	0.4	0.2
Dietary fibre	12.7	4.9
Non-cellulosic polysaccharide	9.0	3.5
Hexose	5.1	2.2
Pentose	2.0	1.4
Uronic acid	1.9	0.8
Cellulose	3.0	1.3
Lignin	0.7	0.5

TABLE 3(g)

DIETARY INTAKES FOR METHANE PRODUCERS (N = 9) AND NON-PRODUCERS (N = 16) IN GROUP 1 SUBJECTS

Food category (g)	Methane producers			Non producers			"t"	
	Mean	1SD	r	MEAN	1SD			
Protein	80	17	-0.55	85	27	0.55	N.S.	
Fat	105	15	-0.13	123	48	1.08	N.S.	
Carbohydrate	232	53	-0.61	288	127	1.25	N.S.	
Energy MJ	9.8	1.6	-0.54	11.0	4.2	0.82	N.S.	
Saturated fat	89	19	-0.63	114	47	1.51	N.S.	
Unsaturated fat	11.5	7.6	-0.11	8.3	6.5	1.11	N.S.	
Cholesterol	0.3	0.1	0.63	0.5	0.3	2.04	N.S.	
Total dietary fibre	17.2	9.8	-0.55	18.5	6.5	0.11	N.S.	

Results were analysed using Student's "t" test and linear regression analysis  
N.S. not significant

TABLE 3(h)

DAILY DIETARY INTAKES FOR METHANE PRODUCERS (N = 34) AND NON-PRODUCERS (N = 22) IN GROUP 2 SUBJECTS

Food category (g)	Methane producers			Non-producers			"t"
	Mean	1SD	r	Mean	1SD		
Protein	70	19	-0.13	63	16	1.43	N.S.
Fat	99	33	-0.12	93	30	0.68	N.S.
Carbohydrate	228	85	-0.24	208	76	0.89	N.S.
Energy MJ	8.7	2.9	-0.20	8.3	2.5	0.53	N.S.
Saturated fat	94	32	-0.28	86	31	0.93	N.S.
Unsaturated fat	5.2	1.7	-0.12	7.3	6.7	1.76	N.S.
Cholesterol	0.4	0.2	-0.00	0.4	0.2	0	N.S.
Total dietary fibre	13.1	5.5	0.22	12.1	4.0	0.72	N.S.
Non-cellulosic polysaccharide	9.3	3.9	0.20	8.6	2.9	0.72	N.S.
Hexose	5.3	2.4	-0.09	4.9	2.1	0.64	N.S.
Pentose	2.3	1.6	0.44*	1.8	1.5	1.19	N.S.
Uronic acid	1.9	0.7	0.11	1.9	0.6	0	N.S.
Cellulose	3.2	1.4	0.18	2.9	1.0	0.90	N.S.
Lignin	0.8	0.6	0.40**	0.7	0.4	0.71	N.S.

Results were analysed using Student's "t" test

\* Using linear regression analysis the concentration of methane excreted correlated with the daily intake of pentose ( $r = 0.44$   $p < 0.01$ )

\*\* The concentration of methane excreted correlated with the daily intake of Lignin ( $r = 0.40$   $p < 0.05$ )

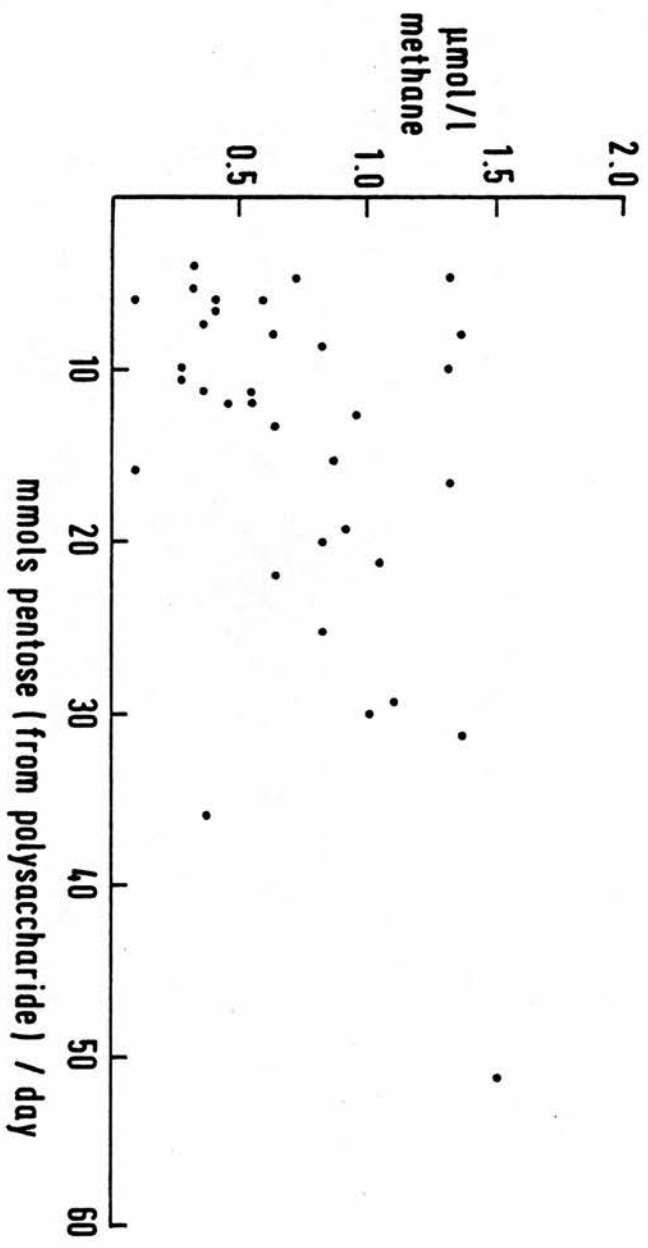


Figure 3(c) Relationship between breath methane concentration ( $\mu\text{mol/l}$ ) and daily intake of pentose (mmols) in 34 subjects. ( $r = 0.44$ ).  $y = 0.02x + 0.47$

TABLE 3(i)

DAILY DIETARY INTAKES FOR NUTRITION EXPERIMENT SUBJECTS (N = 15)

Food category (g)	Mean	1SD
Protein	94	23
Fat	149	46
Carbohydrate	260	110
Energy MJ	11.8	3.8
Total dietary fibre	16.8	6.9
Non-cellulosic polysaccharide	11.8	4.9
Hexose	7.4	2.9
Pentose	2.3*	1.3
Uronic acid	2.2	1.1
Cellulose	4.4	1.7
Lignin	0.5	0.4

\*Within the methane producing subjects (N = 9) there is a correlation between the concentration of breath methane and the intake of pentose ( $r = 0.80$   $p < 0.01$ )

the numbers of subjects is small they have not been subdivided into methane producers and non-producers. However, within the methane excreting subjects (N = 9) there is an association between the concentration of methane excreted and the daily intake of non-cellulosic polysaccharide pentose, ( $r = 0.80$ ,  $p < 0.01$ ).

### FAECAL CHARACTERISTICS

Tables 3(j)(k) contain details of the faecal components and bowel function for group 1 (hospital staff and students) and group 2 (local volunteers) subjects. This data was subdivided into methane producers and non-producers for group 2 subjects (N = 56), Table 3(l). Group 1 subjects were not subdivided since the numbers were small (N = 15).

There were no significant differences between methane producers and non-producers and no associations between the concentration of methane excreted and any faecal characteristic.

Table 3(m) contains details of bowel function and faecal characteristics for the nutrition experiment subjects (N = 15).

Results of logistic regression analysis revealed no associations between the characteristic of methane production and the dietary intake of any food category or the faecal components.



TABLE 3(j)

DETAILS OF FAECAL COMPONENTS AND BOWEL FUNCTIONFOR GROUP 1 SUBJECTS (N = 15)

Faecal component	Mean	1SD
Wet weight g/day	152	60
Dry weight g/day	39	12
Faecal fat mmol/day	16	8
Total bile acids mmol/day	0.88	0.23
Total electrolytes mmol/day	43.42	19.88
Total neutral sterols mmol/day	1.59	0.42
Transit time hrs	50.5	24.0

TABLE 3(k)

DETAILS OF FAECAL COMPONENTS AND BOWEL FUNCTIONFOR GROUP 2 SUBJECTS (N = 56)

Faecal component	Mean	1SD
Wet weight g/day	78	30
Dry weight g/day	21	8
Faecal fat mmol/day	8	5
Total bile acids mmol/day	0.64	0.35
Total electrolytes mmol/day	29.6	14.2
Total neutral sterols mmol/day	1.9	1.0
Transit time hrs	87	35

TABLE 3(1)

DETAILS OF FAECAL COMPONENTS AND BOWEL FUNCTION FOR METHANE PRODUCERS (N = 34)  
AND NON-PRODUCERS (N = 22) IN GROUP 2 SUBJECTS

Faecal component	Methane producer		r	Non-producer		"t"	
	Mean	1SD		Mean	1SD		
Wet weight g/day	79	29	0.19	73	32	0.72	N.S.
Dry weight g/day	22	7	0.18	20	10	0.88	N.S.
Faecal fat mmol/day	8.8	4.4	0.21	8.7	6.2	0.07	N.S.
Total bile acids mmol/day	0.64	0.37	-0.17	0.73	0.45	0.84	N.S.
Total electrolytes mmol/day	32.85	15.47	0.02	29.46	16.99	0.77	N.S.
Total neutral sterols mmol/day	2.00	0.9	0.20	1.8	1.0	0.83	N.S.
Transit time hrs	89	36	0.11	80	35	0.92	N.S.

Results were analysed using Student's "t" test and linear regression analysis  
N.S. not significant

TABLE 3(m)

DETAILS OF FAECAL COMPONENTS AND BOWEL FUNCTION  
FOR NUTRITION EXPERIMENT SUBJECTS (N = 15)

Faecal component	Mean	1SD
Wet weight g/day	141	42
Dry weight g/day	36	8
Faecal fat mmol/day	12	5
Total bile acids mmol/day	1.24	0.64
Transit time hrs	42	14

CHAPTER 4

THE INFLUENCE OF PENTOSE ON BREATH METHANE EXCRETION

The ingestion of foods and administration of dietary fibre components have been shown to influence the excretion of hydrogen in the breath (Calloway 1966; Hickey et al., 1972; Bond and Levitt 1978; Tadesse and Eastwood 1978). Methane production appears to be independent of hydrogen production and is unaffected by the ingestion of substrates (Calloway 1966; Levitt and Ingelfinger 1968; Bond et al., 1971; Tadesse and Eastwood 1978). Recently, contrary to previous studies, the ingestion of lactulose has been shown to increase methane excretion in some subjects (Pitt et al., 1980).

An association between the concentration of breath methane and the daily intake of dietary pentose ( $p < 0.01$ ) and lignin ( $p < 0.05$ ) has been observed in the population study (Chapter 3).

The following experiments were designed to study the effects of ingested pentose-rich foods, pentosan and pentose monomers on methane excretion.

METHODS

Test meals were given to 12 healthy hospital staff or students (8 men, 4 women) aged from 28 to 50 years, mean 35 years, all of whom were methane excreters. All subjects provided a base line end expiratory breath sample after fasting for 12 hours. Following the ingestion of the test meals breath samples were collected every half hour for 5 hours during which time the subjects were involved in their normal working activities. The breath samples were analysed for methane and hydrogen. Previously the pattern of methane excretion whilst fasting for 5 hours was established for all subjects. The time interval between experiments was at least one week.

The test meals given to 4 subjects, contained 5g pentose (approximately twice the average daily intake) and consisted of

1. 385g orange
2. 22.1g coarse bran
3. 200g boiled sprouts or parsnips, 200g boiled carrots and 110g stewed apple.

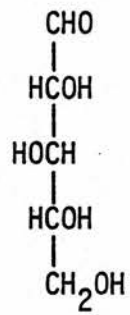
Test meals containing 25g D(+) xylose, 25g L(-) xylose in 250ml water, 20g L(+) arabinose, 20g D(-) arabinose in 200ml water and 10g xylan in 100ml water were given to methane excreting subjects (n = 7, 3, 6, 2 and 3 respectively) (Table 4(a)). 6 non-methane excreting subjects were also given 25g D(+) xylose in 250ml water.

Methane excretion following the test meals is expressed as the mean and standard deviation (n = 10) of the concentrations over 5 hours compared to the fasting base line methane concentration. Methane excretion following the test meals of D(+) xylose and L(+) arabinose were compared to the control period of fasting using Wilcoxon Signed Rank Test.

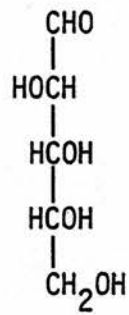
TABLE 4(a)

FORMULAE OF PENTOSES AND XYLAN

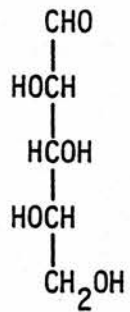
D Xylose



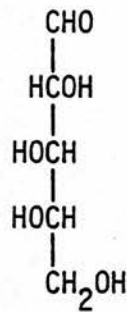
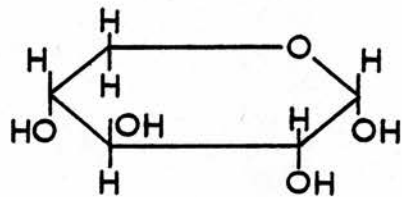
D Arabinose



L Xylose



L Arabinose

 $\alpha$  D Xylopyranose residue

RESULTS

The fasting base line methane concentrations of the 12 subjects ranged from  $0.23\mu\text{mol/l}$  to  $3.15\mu\text{mol/l}$  (mean  $0.97\mu\text{mol/l}$ ). Table 4(b). The base line, mean and standard deviation breath methane concentrations over 5 hours for the control period of fasting and after all test meals are shown in Table 4(b). Test meals of bran, fruit, cooked vegetables and fruit and xylan had no effect on breath methane excretion over 5 hours.

Figure 4(a) shows the base line, 2, 3, 4 and 5 hour breath methane concentrations for each subject after a control period of fasting and following the ingestion of D(+) xylose and L(+) arabinose. After the administration of D(+) xylose and L(+) arabinose there was a significant increase in methane excretion at 90 to 120 minutes compared to base line ( $p < 0.01$ ,  $p < 0.025$  respectively, Wilcoxon Signed Rank Test) and at 210 to 240 minutes compared to the measurement one hour previously ( $p < 0.01$ ,  $p < 0.025$ ).

The range of maximal increases in breath hydrogen ( $\mu\text{mol/l}$ ) above base line for the subjects after the test meal was : fruit ( $n = 4$ )  $0 - 0.36\mu\text{mol/l}$ ; bran ( $n = 4$ )  $0 - 0.81\mu\text{mol/l}$ ; cooked vegetables and fruit ( $n = 4$ )  $0 - 0.41\mu\text{mol/l}$ ; xylan ( $n = 3$ )  $0 - 0.14\mu\text{mol/l}$ ; D(+) xylose ( $n = 7$ )  $0.09 - 0.86\mu\text{mol/l}$ ; L(+) arabinose ( $n = 6$ )  $0.05 - 1.49\mu\text{mol/l}$ .

The pattern of breath hydrogen excretion in methane producers over the 5 hour test period following the ingestion of D(+) xylose and L(+) arabinose was similar to that of methane, i.e. separate peaks at 90 - 120 and 210 - 240 minutes.

The range of maximal increases in breath hydrogen ( $\mu\text{mol/l}$ ) above base line for the non methane producing subjects after ingestion of D(+) xylose ( $n = 6$ ) was  $0.14 - 4.01\mu\text{mol/l}$ .

Both L(-) xylose and D(-) arabinose caused diarrhoea in the methane producing subjects tested, L(-) xylose at 210, 240 and 270 minutes and D(-) arabinose at 80 and 240 minutes. There were no significant changes in methane or hydrogen excretion prior to this.

TABLE 4(b)

BASE LINE (B.L.) MEAN AND STANDARD DEVIATION (S.D.) BREATH METHANE ( $\mu\text{mol/l}$ ) OVER A 5 HOUR PERIOD AT 30 MINUTE INTERVALS

(N = 10) IN CONTROLS (FASTING) AND AFTER INGESTION OF TEST MEALS.

(1) 25g D(+) XYLULOSE (2) 20g L(+) ARABINOSE (3) 10g XYLAN (4) 385g ORANGES

(5) 22.1g COARSE BRAN (6) 200g BOILED PARSNIPS OR SPROUTS, 200g BOILED CARROT, 110g STEWED APPLE.

Subject	Control			D(+) xylose (1)			L(+) arabinose (2)			Xylan (3)			Oranges (4)			Bran (5)			Boiled vegetables (6)			
	B.L.	Mean	S.D.	B.L.	Mean	S.D.	B.L.	Mean	S.D.	B.L.	Mean	S.D.	B.L.	Mean	S.D.	B.L.	Mean	S.D.	B.L.	Mean	S.D.	
1	2.93	2.21	0.23	1.62	3.24	1.44	2.43	3.02	0.95	3.15	2.03	0.59										
2	0.54	0.27	0.14	0.50	0.90	0.18	0.68	0.63	0.18	0.36	0.23	0.14	0.23	0.27	0.09	0.68	0.68	0.18	2.21	1.40	0.36	
3	0.63	0.78	0.36	0.45	1.17	0.41	0.59	0.63	0.14										0.23	0.23	0.09	
4	0.32	0.32	0.05	0.36	0.32	0.09	0.23	0.27	0.09	0.41	0.32	0.09										
5	0.90	0.90	0.18	0.36	0.54	0.14																
6	0.90	0.59	0.18	1.08	1.26	0.59																
7	0.23	0.18	0.05	0.72	1.22	0.36																
8	0.50	0.68	0.14				0.99	2.7	1.35													
9	1.80	0.99	0.23				1.31	1.13	0.18													
10	1.44	1.26	0.32										1.76	1.71	0.32	1.35	1.49	0.18	2.16	1.35	0.32	
11	0.77	0.72	0.09										0.86	0.86	0.14	0.63	0.81	0.23	0.59	0.68	0.09	
12	0.63	0.50	0.14										0.77	0.54	0.14	0.72	0.63	0.18				

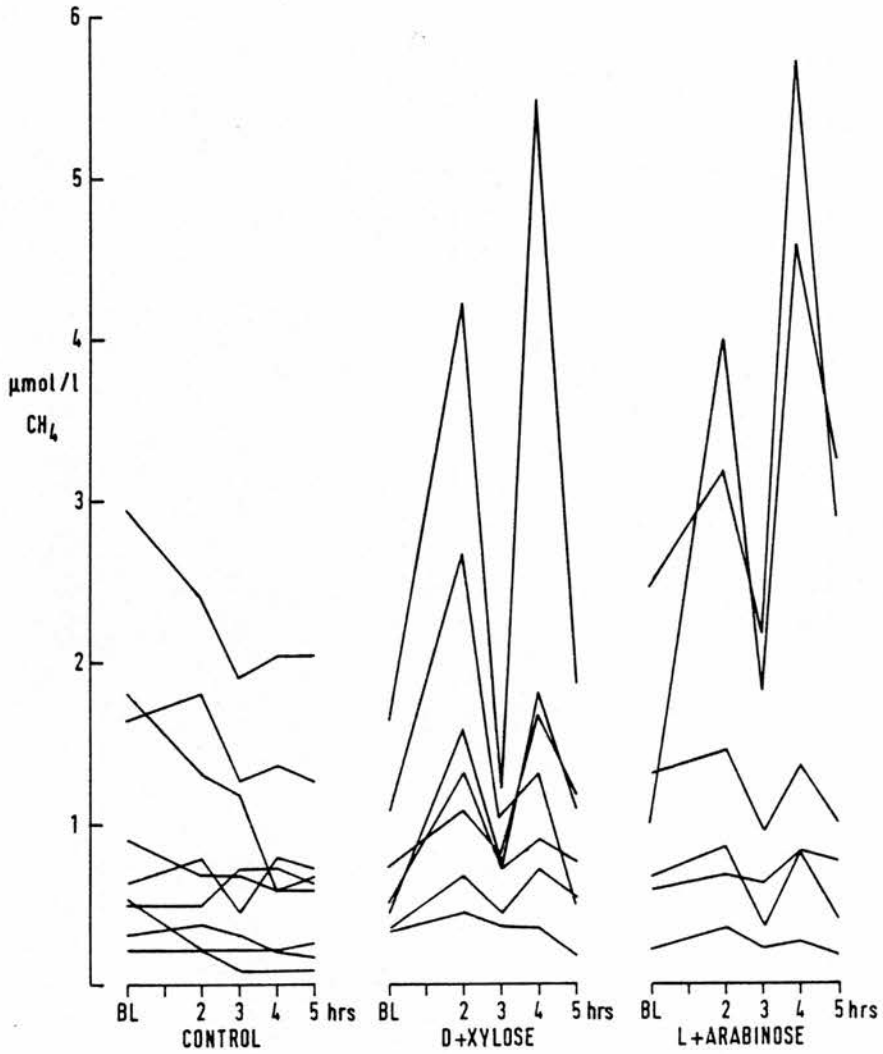


Figure 4(a)

Base line, 2, 3, 4 and 5 hour breath methane concentrations ( $\mu\text{mol/l}$ ) after a control period of fasting and following the ingestion of D(+) xylose and L(+) arabinose.

CHAPTER 5

THE PREVALENCE OF BREATH METHANE EXCRETION IN CLINICAL POPULATIONS

The incidence of breath methane excretion has been studied in various defined clinical groups. Levey and Balchum (1963) showed no associations between methane excretion and a series of clinical conditions. Their patients included 15 on intravenous alimentation or receiving nothing by mouth; 17 patients with constipation or varying degrees of intestinal obstruction; 5 patients at intervals after various operations on the gastrointestinal tract (vagotomy, resection of small intestine, resection of colon, vagotomy and pyloroplasty and closure of bowel wound); 5 patients with cirrhosis, jaundice or hepatic coma. In none of the disorders studied was there a consistent elevation in breath methane concentration. Patients with constipation, on intravenous alimentation, and post-operative had methane concentrations within the normal range as assessed by Levey and Balchum (1963), mean 9 ppm, 11 ppm, 10 ppm and 26 ppm respectively. The concentration of methane excreted by clinical patients was lower than the healthy controls. They had not considered the effects of antibiotics or pre-operative bowel preparations. Haines et al., (1977) have shown an increased prevalence of breath methane excretion in subjects with colonic carcinoma (80%) compared with 39% of subjects with non-malignant large bowel disease and 40% of subjects without large bowel disease.

In the following investigations a wide group of gastrointestinal patients has been studied including patients with Crohn's disease (N = 39), ulcerative colitis (N = 40), irritable bowel syndrome (N = 42) and pneumatosis cystoides intestinalis (N = 9). Many of these patients have altered bowel function and possibly changes in colonic pH and oxygen tension.

The methanogenic bacterium which has been isolated from human faeces (Nottingham and Hungate, 1968) is a strict anaerobe. Conditions

which might alter the colonic environment e.g. oxygen tension, pH, gut transit time and hence the residence time of faeces in the colon and turnover of the methanogenic bacterial population have been investigated.

Two end-expiratory breath samples were obtained from each subject in the following investigations. No subjects were smoking prior to giving a breath sample (Tadesse and Eastwood, 1977). Patients taking antibiotics or laxatives or who had received enemas or intestinal washouts were excluded from these studies. (Karlin et al., 1980b). Patients on elemental diets as part of their pre-operative bowel preparation were also excluded. Patients with intestinal resections were not included unless otherwise stated (see appendix).

The clinical subjects in these investigations have been obtained from various hospitals and departments.

### 1. GASTROINTESTINAL PATIENTS

In-patients and out-patients attending the Gastrointestinal Unit, Western General Hospital 117 men, 147 women aged from 16 to 73 years, mean 49 years.

These subjects were grouped according to their clinical condition. The criteria for clinical diagnosis of these patients are shown in Table 5(a). Figure 5(a) shows the range of methane concentrations in the subjects and in a control patient group. The control patient group (35 men, 29 women aged from 17 to 86 years, mean 48 years) was an age matched group of patients in the general part of the hospital e.g. Urology, Orthopaedics and Gynaecology. The overall percentage of methane producers in the gastrointestinal group was 32% and in the General Hospital group was 53% ( $p < 0.01$ ,  $\chi^2$  test).

Table 5(b) shows the breath methane and hydrogen concentrations for all gastrointestinal and control patients investigated.

TABLE 5(a)

DIAGNOSTIC CRITERIA

Colonic carcinoma Ulcerative colitis Irritable bowel syndrome	Medical history; clinical investigations including haematology and biochemistry, rectal, sigmoidoscopic and barium enema examinations; biopsy
Pneumatosis cystoides intestinalis Crohn's disease	Additional barium follow through examination
Steatorrhoea	Increased faecal fat > 18mmol/day (but not Crohn's disease); Pancreatic function test; jejunal biopsy; tests for bacterial colonisation

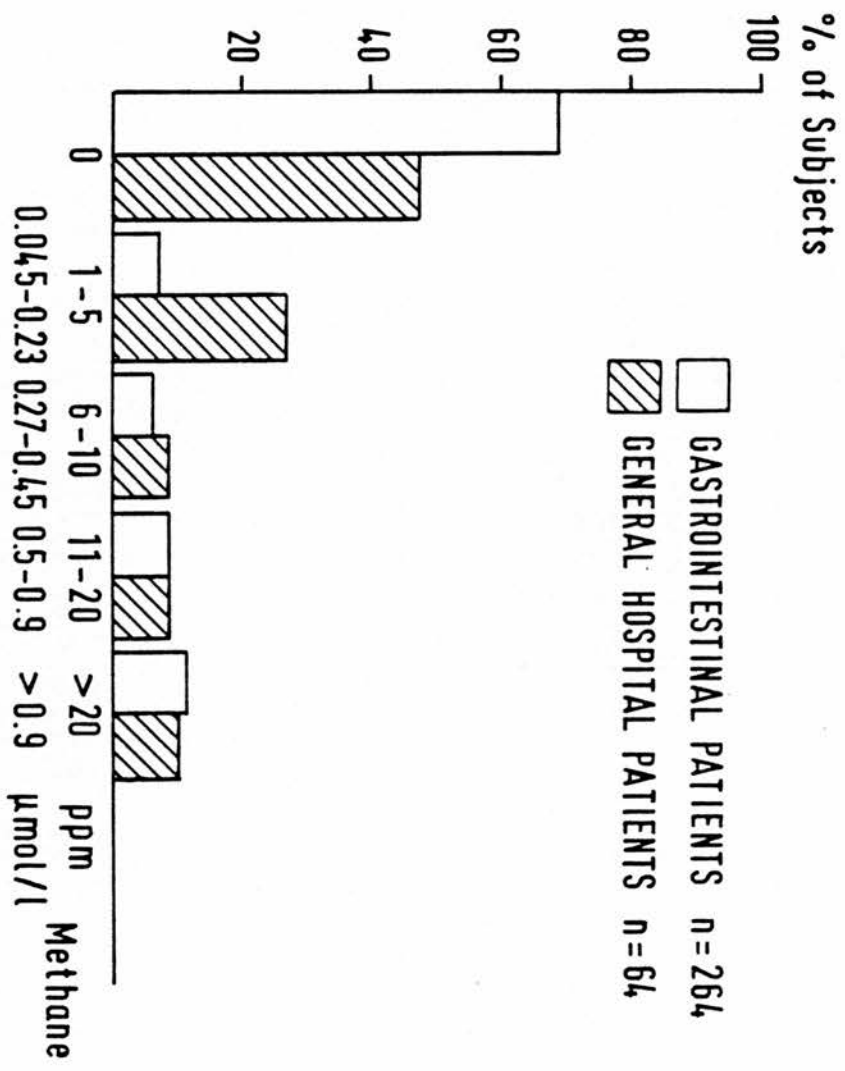


Figure 5(a) Breath methane concentrations ( $\mu\text{mol/l}$ ) in clinical patients

TABLE 5(b)

BREATH METHANE AND HYDROGEN CONCENTRATIONS (RANGE, MEAN AND STANDARD DEVIATION)  
FOR GASTROINTESTINAL AND CONTROL PATIENTS

Disease state	Hydrogen concentration ( $\mu\text{mol/l}$ )	Methane concentration ( $\mu\text{mol/l}$ )		Number of methane producers		p
		Range	Mean $\pm$ 1SD		%	
Colonic carcinoma N = 14	$\leq 0.58$	0.18 - 0.9	0.54 0.27	7	50	N.S.
Crohn's disease N = 39	$< 0.9$ (1.35 1.8, 1.3, 1.84, 1.8)	0.23 - 1.03	0.43 0.33	5	13	$p < 0.001$
Ulcerative colitis N = 40	$< 0.9$ (2.7)	0.2 - 1.08	0.47 0.33	6	15	$p < 0.001$
Irritable bowel syndrome N = 42	$< 0.9$	0.23 - 2.25	0.60 0.52	17	40	N.S.
Pneumatosis cystoides intestinalis N = 9	range 0.31 - 4.14 2.04 $\pm$ 1.21	(1 patient 1.53)		1	11	$p < 0.025$
Steatorrhoea N = 19	$< 0.2$	0.13 - 2.79	1.18 1.12	6	32	N.S.
General gastro- intestinal patients N = 102	$\leq 0.45$	0.09 - 5.4	1.12 1.08	43	42	N.S.
General hospital N = 64	$\leq 0.9$	0.09 - 1.84	0.54 0.45	34	53	

Number of methane producers in each group are compared with the general hospital patients  
N.S. not significant

Within the gastrointestinal group there appear to be variations in the percentage of methane producers with certain diseases. Comparing these gastrointestinal patients with the general hospital patients the significant differences are Crohn's disease ( $p < 0.001$ ), ulcerative colitis ( $p < 0.001$ ), and pneumatosis cystoides intestinalis ( $p < 0.025$ ). There are no significant differences between the general hospital patients and patients with irritable bowel syndrome, steatorrhoea, carcinoma or the remainder of the gastrointestinal patients. This latter group contains patients with lactase deficiency, diverticular disease, post-vagotomy and post-gastrectomy diarrhoea, (see appendix). The percentage of methane producers in this group is 42%.

Breath hydrogen concentrations were low and within normal ranges ( $\leq 0.45 \mu\text{mol/l}$  fasting and  $\leq 0.9 \mu\text{mol/l}$  non fasting) in all subjects except those with pneumatosis cystoides intestinalis ( $2.04 \pm 1.21 \mu\text{mol/l}$ ), 1 patient with ulcerative colitis ( $2.7 \mu\text{mol/l}$ ) and 5 patients with Crohn's disease (1.35, 1.8, 1.3, 1.84,  $1.8 \mu\text{mol/l}$ ), Table 5(b).

Patients with Crohn's disease and ulcerative colitis were subdivided according to the distribution of the disease, (see appendix). 27 patients with ulcerative colitis received Salazopyrin. Of the 6 methane producing patients with ulcerative colitis 3 were on Salazopyrin and 3 were not.

THE INFLUENCE OF SUBSTRATES (GLUCOSE AND LACTOSE) ON BREATH METHANE  
AND HYDROGEN EXCRETION  
IN PATIENTS WITH SMALL INTESTINAL BACTERIAL COLONISATION  
AND LACTASE DEFICIENCY

Patients with diarrhoea and suspected small intestinal bacterial colonisation were given a breath hydrogen test with a glucose test

meal as described below. In situations where anaerobic bacteria colonise the upper small intestine, the bacteria compete for available nutrients, which are fermented anaerobically to produce gases. Measurement of breath hydrogen concentrations and the time relationship of the rise in breath hydrogen following the test meal are useful diagnostic aids for bacterial colonisation. Lactase deficient subjects were identified by their response to a breath hydrogen test with a lactose test meal. On a separate day a jejunal biopsy was taken by means of a Crosby capsule and the brush border lactase activity measured on the mucosal samples (Dahlquist, 1968). All subjects had a lactase activity of  $< 1.0 \mu\text{mol}/\text{min}/\text{g}$  confirming lactase deficiency.

6 methane producing patients with small intestinal bacterial colonisation (positive response to the breath hydrogen test) were given glucose tolerance breath tests. After fasting from 22.00hrs the previous evening the patient gave a breath sample at 09.00hrs by the method previously described. A test meal of 50g D(+) glucose in 250ml water was then given. Breath samples were taken every 15 minutes for the following  $2\frac{1}{4}$ hrs. Table 5(c) contains the results of the glucose breath test which are expressed as base line (BL), mean and standard deviation over  $2\frac{1}{4}$ hrs ( $N = 9$ ) concentration ( $\mu\text{mol}/\text{l}$ ) of hydrogen and methane. The hydrogen and methane concentrations were compared using linear regression analysis and the correlation coefficients ( $r$ ) are shown in Table 5(c). A typical response pattern is illustrated in Figure 5(b). This subject is a high methane excreter but within the normal range. Hydrogen is evolved when the fermentable substrate reaches the bacterial population. Methane appears to be unaltered within the time investigated. The excretion of hydrogen and methane was unrelated (except in one subject  $p < 0.05$ ) in patients

TABLE 5(c)

BASE LINE (B.L.), MEAN AND STANDARD DEVIATION (S.D.) BREATH HYDROGEN AND METHANE CONCENTRATIONS ( $\mu\text{mol/l}$ ) OVER A 2 $\frac{1}{2}$ HR PERIOD AT 15 MINUTE INTERVALS (N = 9)

IN 6 PATIENTS WITH SMALL INTESTINAL BACTERIAL COLONISATION

Subject	Hydrogen			Methane			r	p
	B.L.	Mean	S.D.	B.L.	Mean	S.D.		
1	0.36	3.11	0.9	2.39	1.62	0.14	-0.58	N.S.
2	1.26	3.96	1.08	0.23	0.23	0		
3	0.09	2.16	0.86	1.44	2.16	0.18	0.65	p < 0.05
4	0.14	1.67	1.44	2.07	2.93	0.27	0.32	N.S.
5	0.09	2.34	1.62	0.45	1.35	0.18	0.38	N.S.
6	0.32	0.77	0.68	0.68	0.9	0.14	-0.50	N.S.

Results were analysed using linear regression analysis  
r (correlation coefficient)

TABLE 5(d)

BASE LINE (B.L.), MEAN AND STANDARD DEVIATION (S.D.) BREATH HYDROGEN AND METHANE CONCENTRATIONS ( $\mu\text{mol/l}$ )  
OVER A 3HR PERIOD AT 30 MINUTE INTERVALS (N = 6)  
IN 6 PATIENTS WITH LACTOSE INTOLERANCE

Subject	Hydrogen			Methane			r	
	B.L.	Mean	S.D.	B.L.	Mean	S.D.		
1	0.18	1.53	0.99	2.00	1.45	0.23	-0.5	N.S.
2	0.14	1.44	1.49	0.45	0.36	0.09	-0.64	N.S.
3	0.54	1.08	0.9	1.71	1.58	0.09	-0.09	N.S.
4	0.18	3.87	1.13	0.59	0.59	0		
5	0.9	1.44	0.54	0.23	0.23	0		
6	0.14	1.49	0.36	0.72	0.68	0.05	-0.57	N.S.

Results were analysed using linear regression analysis  
 r (coerrelation coefficient)

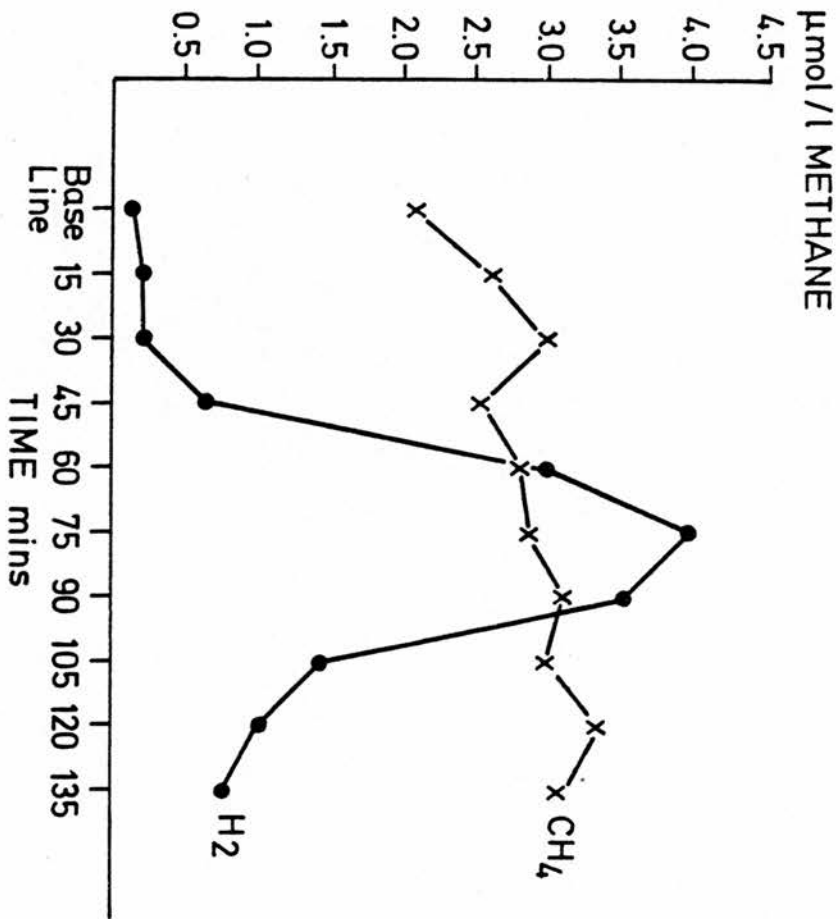


Figure 5(b)

Breath hydrogen and methane concentrations ( $\mu\text{mol/l}$ ) following a 50g glucose test meal in one typical subject.

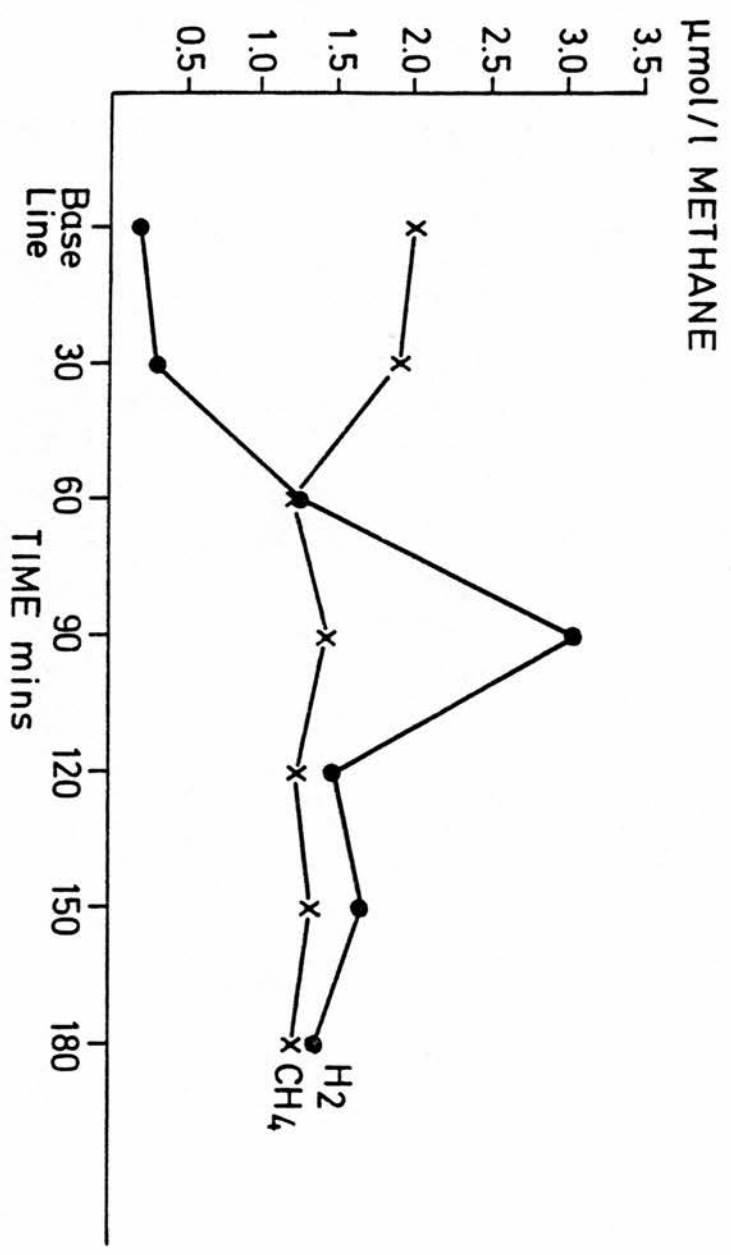


Figure 5(c) Breath hydrogen and methane concentrations ( $\mu\text{mol/l}$ ) following a 50g lactose test meal in one typical subject.

with bacterial colonisation of the small intestine. A similar test was performed with 6 methane excreting lactase deficient subjects. Test meal consisted of 50g lactose in 250ml water and breath samples were obtained every 30 minutes for 3hrs. Table 5(d) contains the results which are expressed as base line (BL), mean and standard deviation over 3hrs (N = 6) concentration ( $\mu\text{mol/l}$ ) of hydrogen and methane. Correlation coefficients (r) obtained by linear regression analysis between hydrogen and methane excreted are shown in Table 5(d). Figure 5(c) illustrates a typical response pattern. There were no associations between hydrogen and methane excretion in these lactase deficient subjects.

## 2. GERIATRIC PATIENTS

66 patients, 17 men and 49 women aged 67 to 98 years, mean 79 years admitted for assessment to geriatric wards of the City and Royal Victoria Hospitals provided breath samples. Routinely plain abdominal X-rays were taken of each patient on the same day as the breath samples. The presence of faeces in the large intestine could be determined from the X-ray. These X-rays were examined by Dr. M. Eastwood and Dr. R. Smith who classified the patients into groups according to the presence of faeces in the caecum shown by stippling in the caecum rather than the presence of gas. Figures 5(d)(e) show X-rays of patients with considerable faeces present in the caecum and with mainly gas in the caecum. Table 5(e) contains the number of methane producers and the concentration excreted in patients with predominantly faeces filled and gas filled caeca ( $p < 0.01$ ,  $\chi^2$  test).



Figure 5(d) Abdominal X-ray of a patient with a faeces-filled caecum



Figure 5(e) Abdominal X-ray of a patient with a gas-filled caecum

TABLE 5(e)

METHANE EXCRETION IN GERIATRIC PATIENTS

	Faeces filled caecum	Gas filled caecum
Number of subjects	43	23
Mean age (years)	79	81
Methane producers (%)	28 (65%)	7 (30%)*
Mean $\pm$ S.D. methane ( $\mu\text{mol}/1$ ) in methane producers	0.92 $\pm$ 0.70	1.49 $\pm$ 0.72**

\* p < 0.01 ( $X^2$  test)  
 \*\* p < 0.005 ("t" test)

Of this patient group 18 were randomly selected for further investigation (10 patients with faecal loading of the caecum and 8 patients with gas-filled caeca).

These patients were given 20 radio-opaque markers of three different shapes (cubes, cylinders and circles) on three consecutive days (day 1, 2 and 3). On days 2, 3 and 4 plain abdominal radiographs were taken to determine the movement of markers along the gut, and the degree of mixing or streaming of the intestinal contents. The first stool passed on day 4 or thereafter was collected to assess total transit time by single stool analysis as described previously. Figures 5(fi,ii,iii) and 5(gi, ii,iii) illustrate the distribution of the radio-opaque markers on days 2, 3 and 4 respectively in a subject with a faeces-filled caecum and a gas-filled caecum. Table 5(f) shows the mean of the total number of pellets counted in the caecum/ascending colon and the descending colon/rectum on days 2, 3 and 4 in the two patient groups (faeces-filled caecum N = 10, gas-filled caecum N = 8).

The mean number of day 1 pellets remaining on day 4 in the caecum/ascending colon was 2 for patients with faeces-filled caeca (N = 10) and 1 for patients with gas-filled caeca (N = 8). The mean number of pellets remaining in the descending colon/rectum on day 4 was 2 for patients with faeces-filled caeca and 7 for patients with gas-filled caeca.

Single stool transit analysis was performed satisfactorily in 7 subjects. A stool collection was not obtained from 7 patients and no markers were found in stools from the remaining 4 patients. Gut transit time assessed by this method gave the following results: 37, 72, 96, 126 and 161 hours for faeces filled caeca patients and 68 and 70 hours for patients with a gas-filled caecum.



Figure 5(f)i

Figure 5(f)i, ii, iii

Radiography of the same subject with a faeces-filled caecum on days 2, 3 and 4 after swallowing 20 radio-opaque markers on days 1, 2 and 3.



Figure 5(f)ii

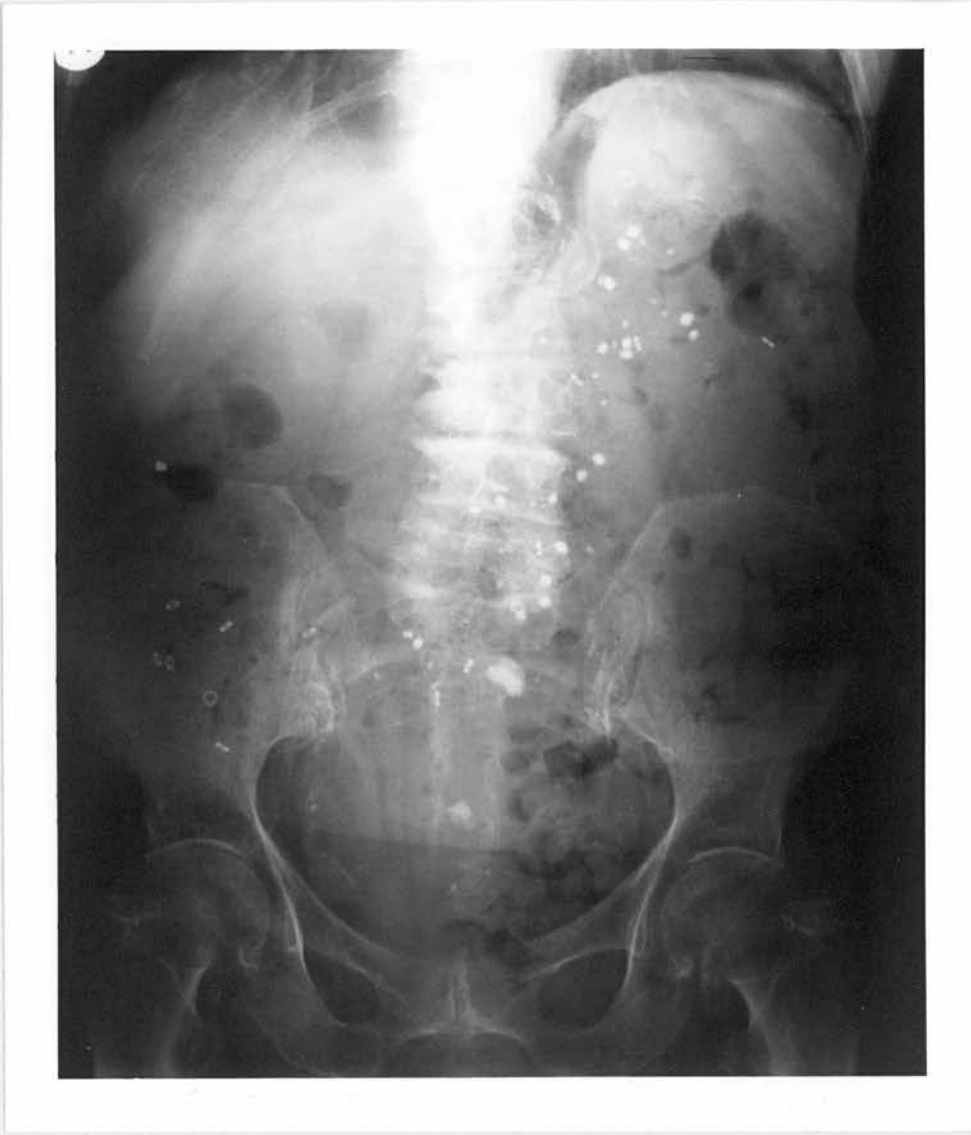


Figure 5(f)iii



Figure 5(g)i

Figure 5(g)i, ii, iii

Radiography of the same subject with a gas-filled caecum on days 2, 3 and 4 after swallowing 20 radio-opaque markers on days 1, 2 and 3.



Figure 5(g)ii



Figure 5(g)iii

TABLE 5(F)

MEAN OF THE TOTAL NUMBER OF PELLETS IN THE CAECUM/ASCENDING COLON  
AND THE DESCENDING COLON/RECTUM ON DAYS 2, 3 AND 4

Day	Faeces filled caecum (N = 10)			Gas filled caecum (N = 8)		
	2	3	4	2	3	4
Ascending colon/caecum	9	12	12	6	5	10
Descending colon/rectum	3	6	10	2	13	19

### 3. AORTO-ILIAC ATHEROSCLEROSIS PATIENTS

60 patients, 44 men and 16 women aged from 45 to 77 years, mean 62 years attending the Peripheral Vascular Clinic, Royal Infirmary of Edinburgh for a routine vascular investigation provided breath samples. Patients were selected for this study on the basis of both a physical examination by a consultant Physician (Dr. E. Housley) on the same day as the breath samples were obtained, and the results of arteriography, either a transfemoral angiogram or a translumber aortogram performed at the Royal Infirmary by Dr. M. Buist, Consultant Radiologist.

The medical history and physical examination involved establishing the claudicating distance, the site of claudication e.g. calf indicates femoro-popliteal disease site, thigh aorto-iliac disease site and the buttock aortic disease site. The patient's smoking status and bowel function were established. Examination included oscillometry; measurement of pulses, femoral, popliteal, dorsalis pedis and posterior tibial; ankle pressure measurement and assessment of skin nutrition.

Patients were subsequently divided into two groups:

1. Aorto-iliac - atherosclerosis primarily affecting the distal abdominal aorta and iliac vessels; patent common and superficial femoral arteries, branches with minimal disease.
2. Femoro-popliteal disease - atherosclerosis primarily affecting vessels distal to the iliac arteries, usually involving the superficial femoral arteries with only minimal disease, if any, observed in the distal abdominal aorta and iliac vessels.

The results of the breath analysis on these patients are shown in Table 5(g). There is a significant difference in the proportion of methane excreting subjects with aorto-iliac and femoro-popliteal disease ( $p < 0.001$ ,  $\chi^2$  test).

TABLE 5(g)

METHANE EXCRETION IN ATHEROSCLEROSIS PATIENTS

	Aorto-iliac disease	Femoro-popliteal disease
Number of subjects	30	30
Mean age (years)	63	62
Methane producers (%)	25 (83%)	9 (30%)*
Mean $\pm$ S.D. methane ( $\mu\text{mol}/1$ ) in methane producers	0.61 $\pm$ 0.51	0.49 $\pm$ 0.31**

\* p < 0.001 ( $\chi^2$  test)  
 \*\* N.S. not significant

CHAPTER 6

THE RAT - AN ANIMAL MODEL

The method for obtaining gas samples from rats using a modified closed circuit apparatus has been previously described, (Chapter 2). Similar techniques have been used to study the effect of feeding cellulose on methane production in Rock Ptarmigan (Gasaway 1976) and of feeding beans on hydrogen production in the rat (Gumbmann and Williams 1971). Bond et al., (1978) studied the influence of high luminal  $PO_2$  on anaerobic bacteria of the rat colon using a similar technique. Absorption of gases from the rat gastrointestinal tract was investigated by measuring pulmonary excretion (Levitt and Levitt 1973). The following investigations involve assessing the influence of various dietary regimes on bacterial metabolism in the rat caecum. Measurements have been made of methane, hydrogen and volatile fatty acid production which are all indices of bacterial metabolism.

The effect of caecectomy and arterial ligation to the caecum on methane production has been studied. Before these experiments were performed, the influence of age and sex on methane excretion in the Wistar rat were evaluated. A previous study (Rodkey et al., 1972) on Spraque-Dawley rats (mean body weight 293g) showed no statistically significant difference between male and female rats with a mean and standard deviation for all rats of  $1.88 \pm 0.88\text{ml CH}_4/\text{kg.hr.}$

## 1. EFFECT OF AGE AND SEX ON METHANE EXCRETION

12 Wistar rats (6 male, 6 female) were maintained from weaning with a routine stock Spratt's diet (details in Chapter 2) and weighed weekly. Growth curves are shown in Figure 6(a). Gas production was tested weekly by the method previously described. Hydrogen was detected from all rats 5 weeks after weaning, ranging from 0.314ml/kg.hr to 0.628ml/kg.hr. Methane was not detected until 9 weeks after weaning i.e. 12 weeks old. The average methane excretion at this age was 0.354ml/kg.hr (males) and 0.464ml/kg.hr (females). The rate of production remained relatively constant over the following 10 weeks of study. The range of methane production in males was 0.27 to 1.60ml/kg.hr and for females was undetectable ( $<0.094\text{ml/rat.hr}$ ) (in 2 rats) to 1.014ml/kg.hr.

A further 14 Wistar rats (8 male, 6 female) less than 12 weeks old were tested and found not to produce methane.

42 Wistar rats (36 male, 6 female) more than 12 weeks old were found to produce methane ranging from 0.184 to 1.748ml/kg.hr (males) and undetectable ( $<0.094\text{ml/rat.hr}$ ) (in 1 rat) to 2.068ml/kg.hr (females). Hydrogen production is known to fluctuate during the day depending on times of food consumption and reaches its maximum 4 hours after feeding (Gumbmann and Williams 1971). Gas samples were taken at different times of the day from the same rat and the variation coefficient found to be 9% for methane. However, since hydrogen was also measured each rat was always tested at the same time of day to eliminate any variations which might be attributed to food ingestion.

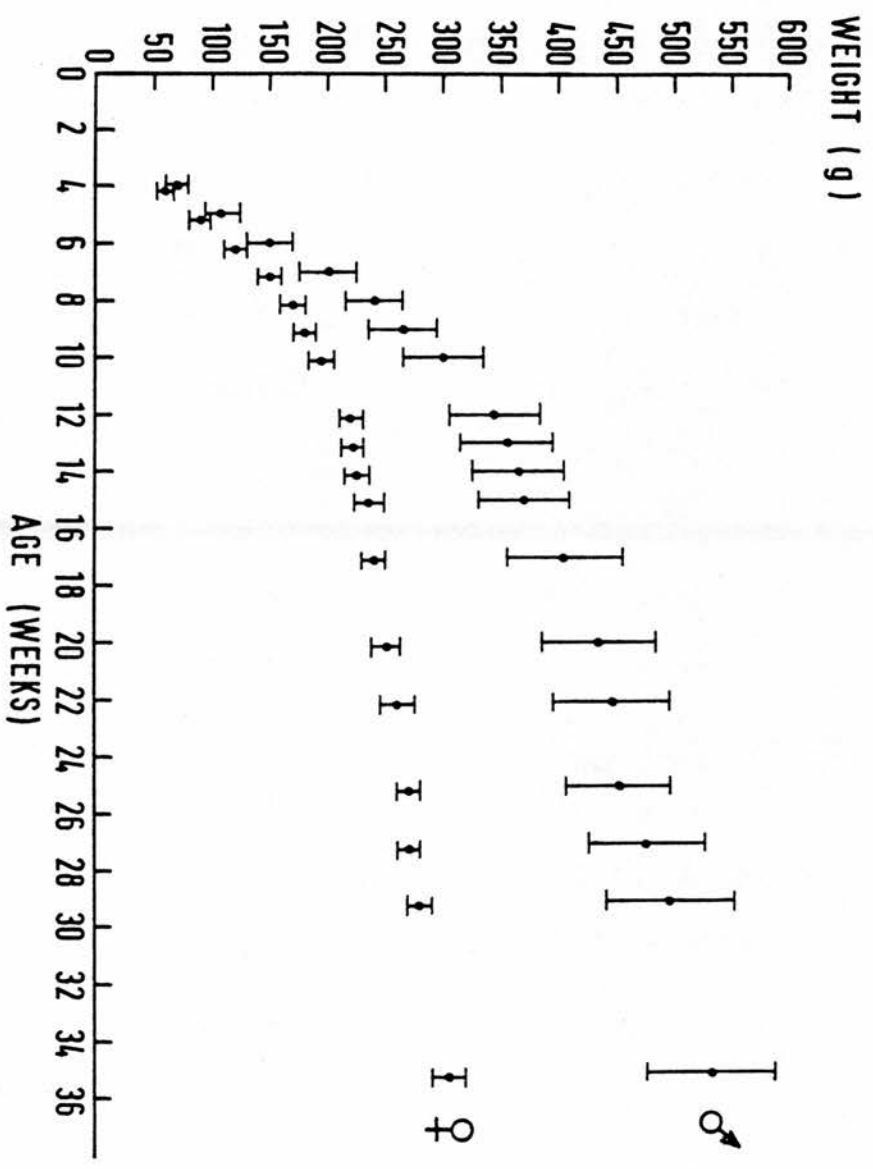


Figure 6(a) Growth curves of male and female Wistar rats

## 2. CAECECTOMY AND ARTERIAL LIGATION EXPERIMENTS

Caecectomy was performed on 5 male Wistar rats more than 3 months old which were shown to excrete methane in readily measurable quantities. This surgical procedure was performed by a surgeon (Hugh Ross). The rats were maintained on a routine stock Spratt's diet and gas analysis was performed at regular intervals up to 8 weeks post-operatively.

Methane was not detected from any animal although hydrogen was present in all rats.

In 2 male Wistar rats, greater than 3 months old previously shown to excrete methane the arteries supplying the caecum were ligated, in 2 male Wistar rats half the arteries were ligated and one animal was given a sham operation. These surgical procedures were also performed by Hugh Ross. One rat receiving "total caecal ischaemia" died of gangrene within 48 hours post-operatively. Gas samples were obtained from the remaining rats up to 8 weeks post-operatively. These results are shown in Table 6(a). The hydrogen production appeared to be unaltered by the surgical procedures. The effect on methane excretion was variable and not significant.

## 3. EFFECT OF AN ELEMENTAL DIET ON METHANE EXCRETION

6 male Wistar rats (3 months old) were fed ad libitum a complete elemental diet (residue free) for 4 weeks. A 24hour faecal collection was made at the start and end of the test period. Gas analysis was performed at the start and at 2 and 4 weeks. The mean stool dry weight per day decreased from  $3.11 \pm 1.54$  to  $0.47 \pm 0.12$ g faeces/day. ( $p < 0.005$ ).

Methane and hydrogen were undetectable in the gas samples at 2 and 4 weeks.

TABLE 6(a)

EFFECT OF ARTERIAL LIGATION ON METHANE AND HYDROGEN EXCRETION. ml/s/kg.hr

	Pre-operative		Post-operative (8 weeks)	
	Hydrogen	Methane	Hydrogen	Methane
Sham operation	0.715	0.306	0.832	0.184
1. Partial arterial ligation	0.544	0.980	0.554	0.924
2. Partial arterial ligation	0.368	0.368	undetectable	0.464
Total arterial ligation	0.435	1.416	0.460	0.184

#### 4. GUM ACACIA SUPPLEMENTATION

Gum arabic is a complex polysaccharide widely used in the foodstuffs and confectionary industry. It has a molecular weight around 850000 and exists as a highly branched molecule with galactose forming its core structure. Arabinose units act as branches on which lie glucuronic acid and rhamnose end units. It is water soluble and highly resistant to acid hydrolysis.

Other workers in the department have investigated the toxicology of gum arabic fed to rats in a long term study. The experiment and results below were part of a detailed investigation into the bacterial metabolism of gum arabic in the rat gastrointestinal tract.

10 Wistar rats (5 male, 5 female) at 3 months of age were fed on gum arabic, incorporated in pellets of reconstituted Oxoid Breeders Diet at a dose of 200 g/kg for 4 weeks. 5 control male rats were fed on the reconstituted Oxoid Breeders Diet alone.

A 24 hour faecal collection was made at the start and end of the test period. Gas analysis was performed at the start and at 2 and 4 weeks. The gas analysis results are shown in Table 6(b). Control rats had no statistically significant changes in methane excretion over 4 weeks. However, the hydrogen decreased significantly ( $p < 0.005$ ) after 4 weeks. The test rats increased methane production significantly over the 4 weeks ( $p < 0.001$ ). There were no changes in hydrogen excretion.

Wet and dry faecal weight increased from  $6.9 \pm 3.68$  to  $10.2 \pm 2.83$  ( $p < 0.05$ ) and from  $3.11 \pm 1.54$  to  $4.06 \pm 0.88$  (NS) respectively.

Volatile fatty acids, acetate, propionate and butyrate were measured in freeze dried faeces. Results in Table 6(c) show the increase

TABLE 6(b)

HYDROGEN AND METHANE EXCRETION IN RATS ON A GUM ACACIA SUPPLEMENTED DIET

	Test rats (N = 10)	Control rats (N = 5)
Hydrogen	Control 1 week	0.69 ± 0.13
	2 weeks	0.29 ± 0.28
	4 weeks	0.36 ± 0.07*
Methane	Control 1 week	0.25 ± 0.22
	2 weeks	0.10 ± 0.01
	4 weeks	0.15 ± 0.05

Results are expressed as mean ± 1SD and are compared using Student's "t" test

\* p < 0.005 compared to control 1 week

\*\* p < 0.001 compared to control 1 week

TABLE 6(c)

INCREASE IN VOLATILE FATTY ACID (mg/g faeces) OVER 4 WEEKS

Volatile fatty acid	Control rats (N = 50)	Test rats (N = 50)	
Acetate	1.93 ± 0.66	6.40 ± 4.27	p < 0.05
Propionate	0.70 ± 0.20	1.67 ± 0.73	p < 0.025
Butyrate	0.85 ± 0.34	1.73 ± 1.23	N.S.

Results are expressed as mean ± 1SD and are compared using Student's "t" test  
N.S. not significant

in volatile fatty acids (mg/g faeces) over 4 weeks in the test and control animals. Test rats have significantly increased concentrations (mg/g faeces) of acetate ( $p < 0.05$ ) and propionate ( $p < 0.025$ ) compared to the control rats.

6 Wistar rats (3 months old) were fed on a complete elemental diet (residue free) containing gum arabic (220g/kg dry weight) in jelly form administered over 4 weeks. Gas samples taken at 2 and 4 weeks contained no methane, although hydrogen was detected at 4 weeks ( $0.981 \pm 0.362$ mls/kg./hr).

#### 5. HIGH FIBRE, SPRATT'S/BRAN AND LOW FIBRE, MEAT/EGG DIETS

The influence of high fibre, Spratt's/bran and low fibre, meat/egg diets on the development and physiology of the large intestine were investigated in 12 Wistar rats (6 male, 6 female). From weaning 6 rats (3 male, 3 female) were fed a high fibre 500g/kg bran incorporated into the routine Spratt's diet (carbohydrate/starch 31g/100g; fat 1.8g/100g; protein 17.8g/100g; crude fibre 5.6g/100g; approximately 915kJ/100g,) 6 rats (3 male, 3 female) were fed a diet of raw minced meat and chopped boiled egg, (fat 12.8g/100g; protein 13.4g/100g; approximately 738kJ/100g. )

Daily food intakes were measured, body weights taken weekly, gas analysis at weekly intervals and a faecal collection taken at 3 and 14 weeks. Faeces were weighed and volatile fatty acids measured. After 3 months the animals were sacrificed, the caecal contents removed and weighed. Caecal volatile fatty acids were measured. Biopsies of liver, jejunum, ileo-caecal region and colon were taken for histology and pathology.

RESULTS

Animals gained weight steadily on both diets with no difference from the control diet of Spratt's alone. Figure 6(a). Daily food consumption reached 30g/day and 40g/day at 12 weeks for the Spratt's/bran and meat/egg fed rats respectively. Methane was not detected in the gas samples from any rat during the experimental period. Hydrogen was detected from all rats (0.63 to 1.88ml/kg.hr). There were no differences in hydrogen production from rats on the two diets.

Table 6(d) shows the volatile fatty acids measured in faeces of 3 week old male and female weanling rats. Although the total faecal volatile fatty acids were not different in the two sexes,  $2.12 \pm 0.46$ , and  $2.87 \pm 0.98$ mg/g in males and females respectively, the females' faeces contained significantly more propionate ( $p < 0.05$ ) and butyrate ( $p < 0.05$ ) than the males' faeces. Mean faecal weights were  $0.564 \pm 0.104$  and  $0.485 \pm 0.115$ g/24hr for female and male rats (3 weeks old).

At 14 weeks the faecal weights were  $25.5 \pm 6.9$  and  $3.1 \pm 0.3$ g/24hrs for the Spratt's/bran and meat/egg fed rats. Faecal volatile fatty acids at 14 weeks are shown in Table 6(e). There were no statistically significant differences in the total volatile fatty acids present in the faeces. However, rats fed Spratt's/bran diet had significantly less propionate ( $p < 0.01$ ) and more butyrate ( $p < 0.05$ ).

A comparison of caecal and faecal volatile fatty acids is shown in Table 6(f). Caecal contents contain a wider spectrum of volatile fatty acids and a higher concentration (mg/g) e.g. total volatile fatty acids 3x, acetate 3x, propionate 4x, isobutyrate 2x, butyrate 6x, isovalerate 5x and valerate 12x the concentration in faeces.

Rats maintained on the Spratt's/bran diet had heavier caeca than rats on the meat/egg diet ( $3.37 \pm 0.33$  and  $1.67 \pm 0.22$ g/100g body weight respectively,  $p < 0.001$ ).

TABLE 6(d)

FAECAL VOLATILE FATTY ACIDS IN 3 WEEK OLD RATS (mg/g)

Volatile fatty acid (VFA)	Male (N = 6) mg/g	Female (N = 6) mg/g	
Acetate	1.94 ± 0.45	2.04 ± 0.25	N.S.
Propionate	0.12 ± 0.12	0.36 ± 0.19	p < 0.05
ISO-butyrate	0	0.08 ± 0.20	
Butyrate	0.06 ± 0.07	0.28 ± 0.20	p < 0.05
ISO-valerate	0	0.06 ± 0.15	
Valerate	0	0.03 ± 0.07	
Total VFA	2.12 ± 0.46	2.87 ± 0.98	N.S.

Results are expressed as mean ± 1SD and are compared using Student's "t" test  
N.S. not significant

TABLE 6(e)

FAECAL VOLATILE FATTY ACIDS IN 14 WEEK OLD RATS (mg/g)

Volatile fatty acid (VFA)	Spratt's/bran (N = 3♀, 3♂) mg/g	Meat/egg (N = 2♀, 2♂) mg/g	
Acetate	5.22 ± 0.76	4.18 ± 0.65	N.S.
Propionate	0.71 ± 0.25	1.42 ± 0.40	p < 0.01
Iso-butyrate	0.01 ± 0.04	0.17 ± 0.06	N.S.
Butyrate	1.28 ± 0.33	0.83 ± 0.23	p < 0.05
Iso-valerate	0	0.24 ± 0.21	
Valerate	0	0.13 ± 0.15	
Total VFA	7.23 ± 1.18	6.99 ± 1.48	N.S.

Results are expressed as mean ± 1SD and are compared using Student's "t" test  
N.S. not significant

TABLE 6(f)

FAECAL AND CAECAL VOLATILE FATTY ACIDS IN 14 WEEK OLD RATS (mg/g)

Volatile fatty acids (VFA)	Caecal VFA mg/g (N = 4♀, 4♂)	Faecal VFA mg/g (N = 4♀, 4♂)
Acetate	12.91 ± 3.99	4.96 ± 0.80
Propionate	3.60 ± 0.88	0.95 ± 0.50
Iso-butyrate	0.08 ± 0.07	0.05 ± 0.08
Butyrate	8.14 ± 4.01	1.19 ± 0.32
Iso-valerate	0.15 ± 0.10	0.03 ± 0.09
Valerate	0.35 ± 0.25	0.03 ± 0.09
Total VFA	25.26 ± 6.01	7.24 ± 1.04

Results are expressed as mean ± 1SD

Histology sections (jejunum, ileo-caecal region, colon and liver) were all normal.

#### DEATHS

During this experiment there were 3 deaths at 3, 10 and during the 12th week of age. Two rats were females and one a male. All three rats were on the meat/egg diet. The most likely cause of death was inspiration pneumonia of unknown origin. The dissected gastrointestinal tracts and lungs were sent to pathology and no abnormalities were reported. Bacterial examination of the lungs revealed no pathogens.

CHAPTER 7

METHANE AND HYDROGEN PRODUCTION  
BY ANAEROBIC BACTERIA OF THE HUMAN INTESTINE

Studies of bacterial methane production have largely concerned methanobacteria (Stadtman 1967; Wolfe 1971; Mah et al., 1977; Zeikus 1977; Wolfe and Higgins 1979). Methanogens are an unusual microbial group (Wolfe 1979) and have proved exceptionally difficult to isolate and culture. The only successful attempt to isolate methanogenic bacteria from human faeces was by Nottingham and Hungate (1968). This bacterium, Methanobacterium ruminantium, utilises hydrogen, carbon dioxide and to some extent formate.

Postgate (1969) has reported methane production by Desulfovibrio sp. and small amounts by Clostridium pasteurianum, a soil and water organism. Production of hydrogen by intestinal anaerobes has been reported by Holdeman and Moore (1977) as a property of certain Clostridial species. A chance finding of methane production by a culture of Clostridium butyricum being examined for hydrogen production prompted further study of methane production by species of anaerobes known to inhabit the human gastrointestinal tract.

MATERIALS AND METHODSSTRAINS

The test strains used were all laboratory isolates from clinical specimens and included: Bacteroides fragilis (7 strains, 694, 696, 697, 698, 700, 702, 710); B. thetaiotaomicron (1 strain); B. ovatus (1 strain, 590); Clostridium perfringens (5 strains, 690, 690X, 701, 713, 714); C. difficile (3 strains, 653, 689, N5); C. fallax (1 strain, 8380); C. paraputrificum (1 strain); C. butyricum (1 strain); C. histolyticum (1 strain, RIE2); C. septicum (1 strain, 558); C. paraperfringens (1 strain, 2227); Anaerobic coccus (7 strains, 941, 359, 6354, 333, 990, 363, 241); Bacteroides sp. (1 strain, 8498); Clostridium sp. (1 strain 977).

CULTURES

All strains were grown in brain-heart infusion broth (Oxoid) in screw-cap bottles half filled with medium and using perforated caps fitted with new rubber seals to facilitate sampling of the headspace gas. Immediately after inoculation the bottles were gassed with oxygen-free nitrogen via a Pasteur pipette and the caps screwed down tightly. For Bacteroides sp. that require carbon dioxide for good growth the bottles were gassed with carbon dioxide before the caps were tightened. All bottles were incubated at 37<sup>0</sup>C, usually for 48hrs.

DETECTION OF METHANE AND HYDROGEN

After incubation, the headspace gas was sampled from each of the culture bottles and analysed for methane and hydrogen by gas chromatography as previously described. The results are expressed as concentration ( $\mu\text{mol/l}$ ).

## REPRODUCIBILITY OF TEST

Two strains, C. histolyticum and C. perfringens were selected and set up in triplicate as described above. Incubation was at 37°C for 5 days and methane and hydrogen were detected as described previously.

## EFFECT OF CARBOHYDRATE SUBSTRATES ON METHANE AND HYDROGEN PRODUCTION

The effect of a range of carbohydrates on methane production by the methane-producing strain of C. histolyticum was investigated. Substrates used were ; D(-) arabinose; L(+) arabinose; cellulose; galactose; D(+) xylose; L(-) xylose; D(+) glucose; gum acacia; raffinose and rhamnose. All were sterilised and incorporated in growth medium to a final concentration of 1%.

Two methanobacteria, Methanobrevibacter ruminantium and Methanosarcina barkeri were used as controls and were grown in methanogen growth medium (Dr. B. Kirsop, personal communication) that was known to support growth of methanogens. These cultures were handled exclusively in an anaerobic cabinet containing 10% carbon dioxide, 10% hydrogen and 80% oxygen-free nitrogen. The Clostridium strain was grown in brain-heart infusion broth as before. Headspace gas was analysed after incubation for 1 week for C. histolyticum and after 3 weeks for the slow growing methanogens. All bottles were set up in duplicate.

## RESULTS

All bacteria produced hydrogen in concentrations ranging from 90 to 18000  $\mu\text{mol/l}$  (Table 7(a)). Most strains of bacteria tested produced no methane or only trace amounts as detected in the headspace

TABLE 7(a)

METHANE AND HYDROGEN CONCENTRATIONS ( $\mu\text{mol/l}$ )  
IN HEADSPACE GAS FROM PURE BACTERIAL CULTURES  
(RESULTS EXPRESSED AS A RANGE FOR SEVERAL STRAINS)

<u>Bacteria</u>	<u>Hydrogen (<math>\mu\text{mol/l}</math>)</u>	<u>Methane (<math>\mu\text{mol/l}</math>)</u>
<u>Bacteroides Fragilis</u> (7 strains)	900 - 1350	0
<u>B. thetaiotaomicron</u>	450	0.36
<u>B. ovatus</u>	90	0
<u>C. perfringens</u> (4 strains) strain 690	15300 - 17550 15300	0 1.08
<u>C. difficile</u> (3 strains)	900 - 7650	0 - 0.36
<u>C. fallax</u>	8100	0
<u>C. paraputrificum</u>	12600	0
<u>C. butyricum</u>	18000	0.18
<u>C. histolyticum</u>	450	1.08
<u>C. septicum</u>	6300	0.9
<u>C. paraperfringens</u>	12600	0.09
<u>Anaerobic cocci</u> (7 strains)	1350 - 7650	0 - 0.45
<u>Bacteroides sp.</u>	1800	0.36
<u>Clostridium sp.</u>	13500	0
Uninoculated medium control	0.36	0

gas. C. perfringens (strain 690) produced 1.08 $\mu$ mol/l methane but the remaining 4 strains produced no methane. Two other Clostridia, a strain of C. septicum (strain 558) and of C. histolyticum (RIE2) produced 0.9 $\mu$ mol and 1.08 $\mu$ mol/l methane respectively (see Table 7(a)).

#### REPRODUCIBILITY TEST

Increased methane production was noted with the test strain of C. histolyticum after five days incubation. The three separate bottles yielded values of 4.32, 4.96 and 5.04 $\mu$ mol/l respectively, (coefficient of variation 8%). Hydrogen values were 135, 180 and 202 $\mu$ mol/l (coefficient of variation 20%). Hydrogen production by the test strain of C. perfringens gave values of 9000, 11250 and 12600 $\mu$ mol/l (coefficient of variation 17%).

#### EFFECT OF CARBOHYDRATE SUBSTRATES

No marked effect on the production of methane was noted when the strain of C. histolyticum was incubated with the various carbohydrate substrates (see Table 7(b)). Production of hydrogen varied with the greatest concentrations obtained with galactose, L. arabinose and gum acacia. The control methanogen strains gave much lower concentrations of methane in the headspace gas in this experiment than were obtained from the cultures when originally received 5,400 $\mu$ mol/l for Methanobrevibacter ruminantium and 36,450 $\mu$ mol/l for Methanosarcina barkeri. Methane concentrations obtained with M. ruminantium were low, ranging from 0.54 to 1.8 $\mu$ mol/l, with the exception of L. arabinose (54.0 $\mu$ mol/l). Increased production of methane from M. barkeri was found with all substrates ranging from 450 to 3600 $\mu$ mol/l.

TABLE 7(b)

METHANE AND HYDROGEN CONCENTRATIONS ( $\mu\text{mol/l}$ ) IN HEADSPACE GAS

AFTER INCUBATION OF TEST STRAIN WITH CARBOHYDRATE SUBSTRATES (MEAN OF TWO SAMPLES)

<u>Substrate</u>	<u>Strain</u>			
	<u>Methanobrevibacter ruminantium</u>	<u>Methanosarcina barkeri</u>	<u>C. histolyticum</u>	<u>C. histolyticum</u>
	<u>Methane</u>	<u>Methane</u>	<u>Methane</u>	<u>Hydrogen</u>
Control (no substrate)	0.9	2.16	0.45	81
D arabinose	0.99	450	1.40	90
L arabinose	54.0	1350	1.40	207
cellulose	1.8	900	1.40	112.5
glucose	0.9	2250	1.67	180
galactose	1.8	1800	1.44	247.5
gum acacia	0.54	900	1.08	202.5
raffinose	0.9	1800	1.17	126
rhamnose	0.68	3600	1.71	144
D xylose	0.72	900	1.04	117
L xylose	0.59	1350	1.35	112.5

CHAPTER 8  
DISCUSSION

## DISCUSSION

Methane is commonly produced in the gut of herbivores and other vertebrates (Hungate 1968; Calloway 1968) and in some invertebrates (Donelan, 1974). Human intestinal tract fermentations produce hydrogen but methane is not a universal component (Levitt 1974). Reports of the proportion of adults excreting methane, measured in the breath, vary from 33% (Bond et al., 1971) to 58% (Pitt et al., 1980). The only report suggesting a clinical significance of excreted breath methane (Haines et al., 1977) states that 80% of patients with large bowel carcinoma compared with 40% of control patients excrete methane. The authors suggest that there are possibly differences in the anaerobic flora of the intestine between patients with carcinoma of the large bowel and normal individuals. The proportion of healthy individuals in a population excreting methane is variable and measurements of breath methane have been little used clinically. Bond et al., (1971) showed that methane excretion was not related to age or sex.

Anaerobic bacterial metabolism in the colon produces hydrogen, methane, volatile fatty acids and carbon dioxide. Hydrogen and methane are excreted in flatus and the expired breath. Of these gut organisms, methanogenic bacteria are the most strictly anaerobic (Smith and Hungate, 1958) and are exceptionally difficult to isolate and culture. Methanogenic bacteria from the gut require a redox potential below  $-0.33V$  at pH7 to initiate growth. Methanobacterium ruminantium has been isolated from human faeces (Nottingham and Hungate, 1968) and is the most common species present in ruminants. This bacterial species utilises hydrogen, carbon dioxide and, to some extent, formate.

In the rumen production of hydrogen and methane are inter-related and depend on the availability of fermentable carbohydrates (Czerkawski

and Breckenridge 1969; Prins 1977; Prins 1979). In man, however, methane production appears to be independent of hydrogen production and is unaffected by the ingestion of substrates (Calloway 1966; Levitt and Ingelfinger 1968; Bond et al., 1971; Tadesse and Eastwood 1978) or by fasting (Levitt and Ingelfinger 1968; Tadesse et al., 1980). Recently, contrary to previous studies, the ingestion of lactulose has been shown to increase methane excretion in some subjects (Pitt et al., 1980). Ingestion of single dose test meals of D(+) xylose and L(+) arabinose have also been shown to increase breath methane excretion (Chapter 4). In a healthy population with a stable dietary intake breath methane concentrations were found to be associated with the intake of the pentose fraction of non-cellulosic polysaccharide (Chapter 3). Finegold and Sutter (1978) suggested that changes in the metabolic activity of intestinal bacteria may take place in response to dietary manipulations without major changes in the number or types of bacteria present. From the bacteriological investigations it is still not clear which bacterium is responsible for methane production in the human colon. Although the large amount of methane produced in the rumen is obviously derived from the metabolism of methanogens, the much smaller amount produced in humans could either result from the metabolism of a small number of methanogens or the production of small amounts of methane by large numbers of other gut organisms such as Clostridia.

Variations in mammalian digestive systems can be related to diet. An important function of the large intestine is microbial digestion which is facilitated by retention in the caecum or in saccular segments of colon. Micro-organisms can convert a high residue diet into more readily utilisable nutrients e.g. volatile fatty acids which are

reabsorbed from the colon. By this process, food which has not been digested in the proximal gut may become a significant source of nutrition. (McBee 1970; Stevens 1978). Herbivores have a capacious rumen or large intestine providing anaerobic conditions, prolonged contact between intestinal micro-organisms and digesta along with a relatively stable neutral pH. Carnivores have a small, minimally segmented gastrointestinal tract which allows brief storage of food in the stomach before a relatively rapid passage through the intestine. Several carnivores, for example, ferrets and hedgehogs have no caecum. Omnivores show great variation in digestive tract structure, for example, the human stomach and small intestine are simple and resemble that of a carnivore, but the large intestine is haustrated throughout its length as in herbivores (Alvarez 1948). The high protein and fat content of the carnivore diet and the relatively low fibre content reduce the necessity for prolonged contact with micro-organisms since there is less need for the nutrients provided by bacterial metabolism. The colonic gas of the dog, the only carnivore investigated, does not contain methane (Calloway 1968). Rural black Africans, with an almost totally vegetarian diet, have a long, wide colon, whereas humans who eat almost no fibre such as Eskimos have a short, straight colon, (Mendeloff 1979). There are no reports on the incidence of methane excretion in these populations.

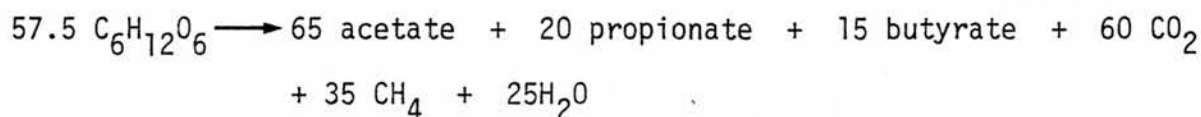
The variation in ability of man to produce methane may reflect a change in colonic microbial flora in response to long term dietary changes, especially the fibre content. A reduction in amount of fermentable substrate in the colon would decrease the necessity for the conversion of hydrogen into methane (4mol hydrogen per mol methane) as a mechanism of reducing the volume of gas.



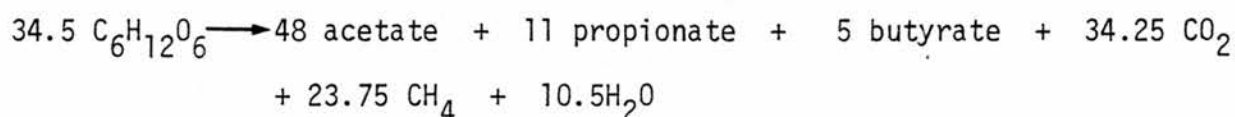
Factors affecting methane formation in man may be different from those in ruminants or in fermenters. A number of factors may be uniting together to bring about methane formation and a single factor such as diet or bacteria may not be the sole determinant.

The fermentation equation for the rumen (Wolin 1960) is similar to the equation for the human large intestine in a methane producing subject. This latter equation has been derived from the concentration of volatile fatty acids in the faeces (Miller and Wolin 1979).

Rumen



Human faeces



Mathematical models may be useful in predicting bacterial mass from the methane yield obtained. Smith and Mah (1978) using Methanosarcina found that the yield per mole of methane was 8.7g when hydrogen/carbon dioxide was the substrate. Methanobacterium formicum yielded 3.5g when hydrogen/carbon dioxide was the substrate (Schauer and Ferry, 1980). Smith and Bryant (1979) estimated the total wet weight of bacteria produced in the large intestine per day to be 4.5g (9.0g dry weight). In a subject excreting about 1 litre of methane per day there could be a yield of around 0.3g methanogen per day. Although subject to some uncertainty this is a substantial bacterial mass.

Investigations reported in this thesis have attempted to identify specific factors influencing methane excretion measured in the breath. The two general questions posed were 1) "Why do all subjects not excrete methane?" and 2) "What determines the amount of methane excreted?".

Formation of methane and hydrogen in ruminants and in vitro cultures are closely related. Although methane was the main gas investigated, hydrogen was also measured in most of the studies reported in this thesis. The method used for gas analysis was that of Tadesse et al., (1979) which combined the use of a katharometer detector with oxygen free nitrogen as a carrier gas. Carbon dioxide and water vapour were absorbed by soda lime and silica gel prior to injecting the gas sample. Use of oxygen free nitrogen as a carrier gas eliminated the huge breath nitrogen peak which would have obliterated the methane peak. The majority of work involved low concentrations of gases and this gas chromatographic system is adequately sensitive and has a wide linear response range. Methane and hydrogen were accurately measured at low concentrations ( $0.09\mu\text{mol/l}$ ) with this method. Pure gases and gas samples with high concentrations of methane and hydrogen, from bacterial cultures, could also be measured by altering the attenuation. Before the development of this method two systems were used : katharometer detector for hydrogen and flame ionization detector for methane. Flame ionization detectors were used for detecting methane by Calloway (1966); Levey and Balchum (1968); Bond et al., (1971); Rodkey et al., (1972) and Pitt et al., (1980). Czerkawski and Clapperton (1968) used a katharometer detector to analyse the carbon dioxide, hydrogen, oxygen, nitrogen and methane content of rumen gas samples. They used argon for the carrier gas and three separating columns with different packings. Similar gases were analysed with a katharometer detector by Whittenbury et al., (1970). When their samples contained methanol, ethanol and acetaldehyde together with methane a flame ionization detector was employed. The responses of these two detection systems were compared using standards with known

concentrations of methane. Although a larger recorder response was obtained with the flame ionization detector, the degree of accuracy as determined by repeated analysis of air and standard gases did not differ significantly for the two systems.

Measurement of breath methane has been used in many other studies (Calloway 1966; Haines et al., 1977; Tadesse and Eastwood 1979; Pitt et al., 1980). Concentrations of methane excreted in breath are very small and so the breath sampling method should collect a large enough quantity for analysis. The method should also reliably reflect the concentration of gases in the alveolar capillary blood. End-expiratory breath samples have been collected by several methods. Calloway (1968) used a rebreathing technique into a multi-laminated plastic bag. Levitt (1969a) put his subjects inside a tight plastic hood, with an oxygen source and carbon dioxide absorber plus an inert gas marker, and collected their breath over a period of time. Metz et al., (1976a) developed and tested a more versatile and acceptable but reliable method based on the Haldane-Priestley tube for measuring alveolar air. The method of Metz et al., (1976a) has been further tested by Bjornekleit and Janssen (1980). They evaluated this breath sampling technique together with two other methods. These two methods were 1) trapping end-expiratory air in a 2000ml plastic bag connected to a three-way stopcock valve and a mouthpiece and 2) trapping end-expiratory air in a polyethylene tube with glass/Teflon valves at both ends. They found the open-ended modified Haldane-Priestley tube to have the smallest coefficient of variation for 10 repeated samples. The authors also studied the effect of hyperventilation on end-expiratory hydrogen concentration and found a mean reduction in pulmonary hydrogen concentration of  $64\% \pm 17\%$ . There is a tendency to hyper-

ventilate in every subject asked to give a breath sample and it is a possible contributory factor to the variation of gas concentrations measured. However, this factor should be constant for each subject and be minimal in subjects familiar with the procedure. A similar tendency was observed during the pulmonary function tests performed on three methane excreting subjects, (Chapter 3). The reductions in methane concentration were 29%, 24% and 23%.

The relationship between intestinal production of hydrogen and excretion in the breath has been investigated by Levitt and Ingelfinger (1968), Levitt (1969a) and Bond et al., (1971). Breath excretion of hydrogen and production in the colon correlate well (Levitt 1969a). Calloway (1968) states that a gas appearing in expired air in as low a concentration as 100ppm is disposed of at a rate approximately 40 to 50ml/hr. Bond et al., (1971) found the rate of pulmonary methane excretion to range from undetectable ( $< 5 \times 10^{-6}$  ml/min) up to 0.66ml/min. They obtained an excellent correlation ( $p < 0.001$ ) between breath methane concentration and the respiratory excretion rate in 22 subjects. Polish (1978) reported the pulmonary excretion of methane to be  $0.45 \pm 0.13$ ml/min. Although pulmonary methane excretion rates were not obtained for all the subjects investigated the results obtained with three subjects are in agreement with those of Bond et al., (1971). Pulmonary methane excretion rates were 0.26, 0.18 and 0.10ml/min. with the corresponding end-expiratory concentrations of 40, 33 and 15 parts per million.

Variation in methane excretion throughout the day was minimal with no characteristic pattern. The coefficient of variation ranged from 15.6 to 33.3%. Food ingestion appeared to have no effect on methane excretion. These findings are in agreement with those of

Calloway (1966), Bond et al., (1971) and Tadesse et al., (1980).

Fasting for 12 hours, which abolishes hydrogen excretion, has previously been shown not to influence methane production (Levitt and Ingelfinger 1968; Tadesse et al., 1980). In the ruminant, fasting decreases methane production with a half life of 24 hours, (Clapperton and Czerkowski 1969). The lack of fermentable substrate decreases the availability of hydrogen which is utilised by the methanogens.

Daily variations in methane excretion were greater than hourly variations ranging from a coefficient of variation of 39.1 to 66.3%. There does not appear to be any pattern to the fluctuations. Calloway (1966) found cyclic variations from day to day in two subjects. She suggested that the fluctuations might reflect a cyclic growth and subsidence of some bacterial strains. No such pattern emerged from the data on six methane producing subjects tested for up to 8 consecutive weeks.

In this thesis the subjects investigated have been divided into two groups according to their methane producing status. Methane producers are those subjects excreting  $>2\text{ppm}$  ( $0.09\mu\text{mol/l}$ ) above atmospheric methane concentrations which are about 2 to 3ppm. Similar grouping criteria have been used by other workers. Bond et al., (1971) in the first systematic investigation performed, defined a methane producer as a subject excreting  $>1\text{ppm}$  above atmospheric concentrations. Non producers excreted  $<1\text{ppm}$  above background concentrations. Haines et al., (1977) also used the concentration of 1ppm to subdivide their subjects. Tadesse et al., (1980) defined methane excreters as subjects excreting  $>0.10\mu\text{mol/l}$  (2ppm). Although they used a slightly higher concentration as the cut-off point they found a similar proportion of their subjects to be methane excreters i.e. one-third, as other workers.

(Calloway 1968; Levitt and Bond 1970; Bond et al., 1971). Recently Pitt et al., (1980) described their non-methane producers as excreting  $< 0.05 \mu\text{mol/l}$  (1ppm) above room air methane concentrations. They comment on the difference in cut-off point between producers and non-producers and reported that 10 of the 256 subjects excreted methane in concentrations between 0.05 and  $0.09 \mu\text{mol/l}$ . In the investigations into prevalences of methane excretion reported in this thesis the concentrations excreted by the subjects are shown. The cut-off point of  $0.09 \mu\text{mol/l}$  above background concentrations was considered to be a reliable, accurate concentration within the detection capacity of the gas chromatograph system used.

#### Factors influencing methane excretion in non-clinical populations.

The proportion of methane producers in the hospital staff and student population (33%) is in accord with previous findings of normal populations (Bond et al., 1971; Haines et al., 1977; Tadesse et al., 1980). However, the proportion of methane producers in the local volunteer population (70%) is considerably higher. Within the two groups there were no statistically significant sex differences. There were no differences in the range of methane produced between the two groups. Within the local volunteer group 34 married couples were investigated. They did not show any degree of spouse concordancy. The methane status of children and parents conformed with previous observations by Bond et al., (1971) although the numbers were small. These workers suggested that the familial tendency could be due to early environmental rather than genetic factors, since a large group of unrelated children who were housed together from an early age all had the same methane producing status. The majority of adult subjects provided breath samples 3 times at 6 month intervals. Their breath methane concentrations only varied

by a maximum of 5ppm. This was reassuring when breath methane concentrations were correlated with dietary intakes. An obvious difference between the two groups is the age distribution. This might cause the difference in methane prevalences. Within both groups there were no associations between age and methane excretion. To check this observation extra young local volunteer subjects (N = 15) were recruited. Methane excretion was measured in 80% of these subjects.

Within the two groups other differences were identified which might influence methane production. Studies which associate intestinal flora with dietary intake are conflicting (Gorbach et al., 1967; Hill and Drasar 1975; Bornside 1978; Finegold and Sutter 1978; Moore et al., 1978). There are, however, suggestions that changes in bacterial populations or the metabolic activity of bacteria might occur with long term dietary manipulations (Finegold and Sutter 1978).

The usual diets of all subjects were investigated with relation to methane excretion. Diet intakes were assessed by a dietician using methods previously described. Although food intakes were not weighed the method was considered to be accurate. A daily visit to the subjects contributed to this accuracy. Results of the dietary intakes of the local volunteers were similar to those obtained by Bingham et al., (1981). They studied the dietary intakes of 63 subjects aged 20 to 80 years living in a Cambridgeshire village. These workers calculated the dietary intakes for men and women respectively to be; energy MJ  $10.02 \pm 2.41$ ;  $8.18 \pm 2.08$ , fat (g)  $104.2 \pm 27.3$ ;  $89.8 \pm 27.1$ , carbohydrate (g)  $285.4 \pm 81.1$ ;  $228.8 \pm 74.2$ , protein (g)  $77.3 \pm 20.3$ ;  $67.3 \pm 15.6$ , fibre (g)  $20.1 \pm 5.4$ ;  $19.8 \pm 5.3$ . Their method included weighing and recording food intake. The results of this study and that of Bingham et al, (1981) show large ranges in the intake of almost every nutrient. When comparing the dietary intakes of the Scottish

and English subjects regional variations have to be considered. Dietary intakes in the hospital staff and student population were all higher than the local volunteers, a finding which probably reflects the age distribution. No differences in diet intake were found between methane producers and non-producers in either population group. However, within methane producers in the local volunteer group there was an association between methane concentration and pentose ( $p < 0.01$ ) and lignin intake ( $p < 0.05$ ). An association was also found between methane excretion and pentose intake in the nutrition experiment subjects.

Unavailable carbohydrates as defined by McCance and Lawrence (1929) are identical with the term "dietary fibre" introduced by Trowell (1972). This includes pectins, hemicelluloses, celluloses and lignin and is an adequate classification for nutritional purposes, although more detailed chemical classifications are used for analytical purposes. The definition of dietary fibre used by Cummings (1976) is "dietary fibre is a group of substances of plant origin, which are found largely but not entirely in the plant cell wall and which are thought to be neither digested nor absorbed in the upper gastrointestinal tract". Southgate et al., (1976) define dietary fibre as, "the sum of the polysaccharides and lignin that are not digested by the endogenous secretions of the gastrointestinal tract". The hemicellulose fraction of dietary fibre is made up of hexoses, pentoses and uronic acid residues which are metabolised differently, (Touster, 1975). Values for the fibre content of foods have been taken from Southgate (1969) and Southgate et al., (1976). Analysis of the components of dietary fibre in terms of their constituent monosaccharides involves a procedure of sequential extraction and hydrolysis. The residue insoluble in alcohol gives non-cellulosic polysaccharide as component hexoses, pentoses

and uronic acid, cellulose as glucose and lignin as the residue insoluble in 72% W/W  $H_2SO_4$ , (Southgate, 1969).

Hemicellulose is metabolised by bacteria as it passes through the human intestine to a greater extent than cellulose. Values for the amount digested vary, for example  $56\% \pm 29\%$  (Williams and Olmsted 1936) and 87.2% (Southgate and Durnin 1970) for hemicellulose. In humans there seems to be some relationship with the subjects age, older people digesting hemicellulose more completely (Hummel et al., 1943; Southgate and Durnin 1970). Dietary pentose is the major non-cellulosic polysaccharide of bran, wholemeal flour and certain fruits and vegetables (Southgate et al., 1976). The xylose-arabinose ratio varies with the species and maturity of the plant. In bran the hemicellulose arabino-xylan has equal proportions of arabinose and xylose. Properties of hemicellulose include water holding capacity, digestibility, the capacity to bind ions (Eastwood and Mitchell, 1976) and the ability to increase faecal weight (Cummings et al., 1978). Plant polysaccharides are degraded by colonic bacteria (Vercellotti et al., 1978; Salyers 1979) and it has been suggested that the majority of hemicelluloses are digested in the small bowel by normal bacterial flora (Holloway et al., 1980). In ruminants, the digestibility of hemicellulose depends on the source and physical nature of the fibre, and is inversely related to the lignin present (Gordon 1978).

The mean daily pentose intake in the 34 methane excreting subjects was 2.4g and the mean daily methane excretion rate was 0.2 litres. This gives a relationship of approximately 1mole methane yielded from the fermentation of 2moles pentose. In ruminants, (Hungate, 1968) there is an observed yield of  $0.42\mu\text{mols}$  methane for each  $\mu\text{mol}$  of food digested. Considering the limitations of the methods, for example

diet analysis and methane production rates the molar methane yields for subjects on regular diets closely resembles methane yields in the rumen. The association between breath methane concentration and the estimated intake of pentose containing fibre may be the result of a steady metabolic state in the caecum.

Ingestion of test meals of pentose rich foods did not lead to any significant increases in breath methane excretion over 5 hours. Xylan, from larchwood, has a molecular weight of around 20,000 and consists of  $\beta$  1 - 4 linked xylopyranose residues. Xylan ingestion did not alter methane excretion. There was, however, an increase in hydrogen excretion following the bran test meal, up to  $0.81\mu\text{mol/l}$  in some subjects, an observation which has been made previously, (Bond and Levitt 1978). This response could reflect the metabolism of retrograde starch which is held within the bran matrix and carried to the distal intestine. Ingestion of 10g hemicellulose or 20g lignin did not alter methane excretion over 6 hours (Tadesse and Eastwood 1978). The lack of gas production following acute complex polysaccharide administration could be due to a relatively slow metabolic response of colonic bacteria. When pentoses were taken in monomeric form D(+) xylose and L(+) arabinose, significant increases in breath methane were observed during periods at 1.5 to 2 hours and 3.5 to 4 hours after ingestion. The first peak of the biphasic methane response is probably caused by the fermentation of the unabsorbed fraction of pentose (approximately half the dose) passing into the caecum. The reason for the two increases in gas excretion is uncertain but may result from the activity of two different methanogenic bacterial species reflecting the complexity of bacterial metabolism in the caecum. After administration of D(+) xylose, breath hydrogen concentrations

were less in methane producing subjects compared with non-producers (0.09 - 0.86 $\mu$ mol/l and 0.14 - 4.01 $\mu$ mol/l respectively). In methane excreters following pentose ingestion hydrogen may be utilised in the conversion of carbon dioxide to methane. Greater increases in methane excretion were observed in subjects who had higher base line concentrations. This may reflect a larger methanogenic bacterial population. The production of methane after the acute administration of free pentoses and the absence of methane production after the ingestion of polysaccharide sources may indicate that the release and availability of free pentose monomers from plant polysaccharides may be rate limiting steps in this process.

Assuming that 12g of the 25g D(+) xylose test meal reached the fermentative bacteria the yield of methane to pentose for the subject with the largest response is 1mole methane from 20moles pentose. This was probably a saturation dose for the bacterial enzymes and it would be interesting to note the methane excretion response to smaller xylose doses.

There were no statistically significant differences in faecal components or bowel function between methane producers and non-producers. The concentration of methane excretion was not related to any faecal characteristic. Bacterial populations will only become established in the gut if the retention time is sufficient to allow at least one generation to occur before the passage in the faeces. Methanogenic bacteria are slow growing and hence require a longer retention time (Mah et al., 1977). In this study there were no differences in stool weight or gut transit time with methane producing status. Levey and Balchum (1963) and Calloway (1966) did not find an association between methane production and bowel habits. Levitt and Duane (1972) however, found a correlation between methane production and stool buoyancy.

They proposed that stools float chiefly on the basis of their methane content and not on the basis of their fat content.

Factors influencing methane excretion in clinical populations.

Clinical problems related to intestinal gas include repetitive eructation, abdominal discomfort and bloating, excessive passage of flatus, acute gastric dilatation, post-operative "gas-bloat" syndrome, intestinal gas and bowel obstruction and gas explosions in the colon (Bond and Levitt 1975; Levitt et al., 1976; Polish 1978; Levitt and Bond 1980; La Brooy et al., 1981).

Haines et al., (1977) reported a higher proportion (80%) of methane excretors with colonic carcinoma than control patients (40%). However, Karlin et al., (1980a) in a study on 20 carcinoma patients, did not support these findings. Unfortunately, the number of carcinoma patients investigated in this study (N = 14) is too small to give accurate support to either previous report. Further patients require to be investigated. There were significant differences in the prevalence of methane excretors in patients with chronic diarrhoea states, for example, colitis, Crohn's disease and pneumatosis cystoides intestinalis. These patients are likely to have a predominantly rapid colonic transit time. Medication, for example, Salazopyrin did not appear to influence methane excretion in patients with ulcerative colitis. There is no information regarding bowel function such as stool weight or gut transit time for these gastrointestinal patients. However, variation within the normal range, as previously described, did not influence methane excretion. Alterations in these patients' diets, for example, considerably reduced dietary fibre intake may contribute to the low prevalence of methane excretion. Dietary information may be of use in future investigations of this nature. When Crohn's disease

and ulcerative colitis patients are further grouped according to the extent and distribution of the disease (see appendix) the numbers are reduced so as to make interpretation of the results increasingly speculative.

The overall prevalence of methane excretion in patients with steatorrhoea was not significantly different from the control patients suggesting that the presence of excess fat in the colon does not influence methanogenesis.

No patients investigated had received enemas prior to the breath test. Colonoscopy preparation including liquid diet, laxative and tap water enemas have been shown to reduce breath hydrogen and methane excretion by 90% (Karlin et al., 1980b). They only investigated 4 methane producers, 2 subjects returned to  $\geq 100\%$  base line by day 3 to 5 and 2 subjects had prolonged abolition of breath methane.

In a study of 301 apparently healthy subjects (Thompson and Heaton, 1980) 13.6% were shown to have typical symptom patterns of the spastic irritable bowel syndrome; 7% suffered non-colonic pain (heart-burn); 3.7% had painless diarrhoea not associated with irritable bowel syndrome and 6% had painless constipation. They observed that 4 clinically functional bowel syndromes existed in almost 1/3 of the subjects studied. Ferguson et al., (1977) showed that 26% of gastrointestinal patients attending outpatient clinics had irritable bowel syndrome and 31% for all so-called "functional" disorders. In this study 16% of all gastrointestinal patients (N = 264) had irritable bowel syndrome.

Since there is a wide normal range of breath methane concentrations an individual's methane status appears to have little clinical application.

Breath methane concentrations in subjects with small intestinal bacterial colonisation did not alter following ingestion of a glucose test meal. Methanogens appear not to be involved in bacterial colonisation and not to utilise the hydrogen evolved by other bacteria within the period of investigation. Similarly, the presence of lactose in the colon of lactase deficient subjects did not influence methane production. Evolution of hydrogen and methane were unrelated. Breath hydrogen responses in these patients were similar regardless of their methane-producing status. Perman et al., (1981) suggested that the hydrogen response to non absorbable carbohydrates may be altered in methane producers. They investigated the influence of pH on hydrogen excretion in non-methane producing subjects only. In vivo inhibition of hydrogen production following lactulose or lactose administration with acidic conditions was observed when colonic contents were acidified by addition of lactulose to the diet for 1 week (0.3g/kg twice daily, 20 - 40ml).

The overall proportion of methane producers in the elderly patients was 53%. When these subjects were grouped into those with a caecum mainly filled with faeces and those with a caecum mainly filled with gas the proportions were 65% and 30% respectively. ( $p < 0.01$ ). The two groups were age matched. It is likely that the methane producing status of patients could be altered with the amount of faeces present in the caecum. A slow colonic transit time may create a different bacterial environment to that found with a rapid transit through the colon. An attempt to measure the residence time in the caecum was made using pellets to assess the degree of retention present. Methods of measuring the time that food residue takes to pass through the bowel are based on the appearance in the stool of a marker given in a single dose.

The effect of mixing is not considered when interpreting the results (Cummings et al., 1976; Smith and Eastwood 1980). The main value of pellets depends on how closely they mimic the natural dietary residue. Wiggins and Cummings (1976) suggest that in part of the gut a 'pool' of residue exists in which mixing occurs and that this region is probably the caecum and right colon. Cummings and Wiggins (1976) describe a method for measuring transit through the gut by analysis of a single stool. They state that once transit times exceed four days, then the accuracy decreases. In a population where the average transit time is of the order of six days or more, such as constipated subjects, then the selection of a later stool is required. In the elderly patients investigated there appeared to be an accumulation of pellets in the right side of the colon in patients with a faeces filled caecum and an accumulation of pellets on the left side in patients with a gas filled caecum. However, the pellets did not appear to mix with the colonic contents and so the observations merely relate to the behaviour of the pellets and not necessarily to the colonic contents. The impression of the radiologist while studying three consecutive X-rays of the patients with a caecum mainly faeces filled was that the caecum remained full of faeces and the pellets moved around the mass. Clearly, the method used to assess faecal mixing or streaming was not successful.

However, under the conditions of this study the two patient groups did behave differently. Results of the single stool analysis of transit time were variable. The prevalence of methane excretion in an age matched group of non-hospitalised healthy subjects from the Muirhouse/Pilton area (70%) was not significantly different from patients with a faeces filled caecum. This is likely to be the more common condition in elderly subjects.

Patients with atherosclerosis of the lower abdominal aorta and iliac vessels had a higher prevalence of methane production compared to those with atherosclerosis of the femoral and popliteal vessels (83% and 30% respectively,  $p < 0.001$ ). The diagnostic criteria were described in Chapter 5. A control group of age matched patients attending clinics other than the Peripheral Vascular or Gastrointestinal clinics at the Royal Infirmary was not obtained. If the results of this study are compared with a non-hospitalised age matched group of subjects from the Muirhouse/Pilton area of Edinburgh (Chapter 3) with an age range of 49 to 74 years, mean 64 years, ( $N = 30$ ) and 67% methane producers, then there is a statistically significant difference between the femoro-popliteal disease group and the controls ( $p < 0.01$ ) but no difference between the controls and the aorto-iliac disease patients. In view of the large variations in the prevalence of methane production in normal control populations the particular control group used is likely to be important. The catchment area for patients attending the Peripheral Vascular Clinic is wide (approximately a 25mile radius for the patients in this study) whereas the control subjects came from a small area within Edinburgh. Varying degrees of occlusion of the visceral arteries are quite commonly encountered in normals over the age of 45 years and often without any demonstrable effect upon their health. A half to one third show some narrowing of the coeliac axis and/or superior mesenteric artery which supplies the right colon. When arteriosclerotic changes are present elsewhere in the body, the figure rises to between half to three quarters. The coeliac axis and superior mesenteric artery are affected in roughly equal proportions, the inferior mesenteric artery rather less so. Stenosis and occlusions of the visceral arteries are common lesions. Due to the capacity for

building collateral flow around a blocked visceral artery, the incidence of asymptomatic stenosis is extremely high. Functional impairment may occur before there is any discernible anatomical change, (Marston 1977). A higher incidence of methane production in these patients may indicate a diminished colonic circulation.

It is not clear from these clinical investigations whether methane production causes or is a result of certain conditions associated with higher methane production prevalences or indeed, whether the absence of methane production is a predisposition to certain gastrointestinal diseases. Results of methane status may only be significant in population studies. It is unlikely that an individual's methane status could be used as a diagnostic aid. There do not appear to be any significant differences between the concentrations of methane excreted by clinical patients with varying disease conditions and healthy subjects.

Methane production and animal models. In rats methane production was not related to sex although methane production was undetectable ( $<0.094\text{ml/hr}$  for a 200g rat) in rats less than 3 months old. In humans, production of methane is not detected in children less than 2 years and the adult excretion pattern is not established until 10 years, (Bond et al., 1971). There may be a critical period during which time methanogenic bacteria are able to colonise the gastrointestinal tract (Savage 1978). Colonisation may only occur in the mature gastrointestinal tract if the colonic conditions are considerably altered. Both caeectomy and the feeding of an elemental diet to intact rats abolished methanogenesis although hydrogen production still occurred following caeectomy. Methanogenic bacteria appear to colonise the caecum of the rat and require a fibrous residue, possibly to assist colonisation

and as a substrate. The polysaccharide, gum arabic fed at a dose of 200g/kg (approximately 6g/day) significantly increased methane excretion ( $p < 0.001$ ) although hydrogen excretion was not significantly altered. This result is of particular interest in view of the relationship found between methane excretion and the long term ingestion of the pentose fraction of non-cellulosic polysaccharides (Chapter 3) and the acute administration of pentose monomers (Chapter 4) in human subjects. A gum arabic supplemented elemental diet abolished methane production. It can be concluded that gum arabic degradation occurs in the caecum and is associated with increased methane excretion, increased volatile fatty acid concentration and changes in the proportions of various volatile fatty acids in the faeces. Changes in caecal environment brought about by an elemental diet alter these relationships with gum arabic metabolism.

Results obtained from feeding meat/egg and Spratt's/bran diets are in agreement with those of other workers using high and low fibre diets (Ryan et al., 1979; Murray et al., 1980). These workers state that the large intestinal mass is related in part to the amount of dietary fibre and that long-term feeding of a fibre-free diet leads to atrophy of the large intestine while a high-fibre diet leads to its hypertrophy. There is also a similar but less pronounced effect on small intestinal mass. Methane excretion was undetectable from rats on both dietary regimes although the rats were greater than 3 months old. An increased fibre content in the diet increases the rate of gastrointestinal transit time. This effect may have prevented the colonisation of the gut by methanogens or other bacteria further back in the bacterial chain. A lack of fibrous residue may also prevent bacterial colonisation in the large intestine. Changes in

diet, for example, gum arabic supplementation and a meat/egg diet are associated with changes in the faecal volatile fatty acid concentrations and a change in the proportion of various volatile fatty acids in the faeces. Similar observations have been made in the pig (Cranwell 1968), in rodents (McBee, 1970) and in the dog, pig and pony (Stevens, 1977). Stevens (1977, 1978) reported that the mean concentrations of volatile fatty acids in the large intestines of dogs on a meat diet, pigs on a grain diet and ponies on a low fibre pelleted diet were equal to or greater than those found in the ruminant forestomach. The total volatile fatty acid concentrations were little affected by feeding higher fibre diets to those species. The nutritional value of organic acids depends on the actual quantity absorbed by the animal. Total absorption of volatile fatty acids from the digestive tract of cattle has been estimated to provide 70% of the animal's basal energy requirements whereas volatile fatty acids produced in the pony's large intestine supply a minimum of 25% of the animal's basal energy requirements (Stevens 1977).

Methane and hydrogen production by anaerobic gastrointestinal bacteria. It is well known that certain anaerobes produce hydrogen but little quantitative data is available (Holdeman and Moore 1977). Of the organisms tested in this study hydrogen was produced in largest amounts by strains of the genus Clostridium. Smaller concentrations were produced by anaerobic cocci and the smallest by members of the genus Bacteroides. Species and even strain variations were however apparent. This hydrogen may play a part in the pathogenesis of several intestinal disorders such as chronic idiopathic intestinal pseudo-obstruction (Holbrook and Cartwright 1981); pneumatosis cystoides intestinalis (Yale 1975; Van der Linden and Hoflin 1978; Van der Linden

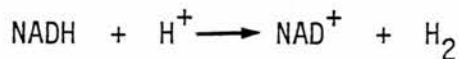
and Marsell 1979) and possibly necrotizing enterocolitis (Howard et al., 1977). The reasons why accumulation of hydrogen gas occurs in, or causes certain conditions is an intriguing problem meriting further study.

Small but significant amounts of methane were produced by three test strains (C. histolyticum, C. perfringens and C. septicum). The only other members of the genus Clostridium known to produce methane is C. pasteurianum, a soil and water organism (Postgate 1969). Methane production was a reproducible finding in strains where it was detected but was not a constant finding within all strains of any particular species.

Attempts to compare methane production from known methanogens with the test strains showed similar amounts of methane from Methanobacterium ruminantium but much more methane was produced by Methanosarcina barkeri. The amounts of methane obtained with these methanogens were less than that obtained by other workers, (Dr. B. Kirsop, personal communication) and less than that recorded from the original cultures of these methanogens. The inability to produce methane concentrations corresponding to those of more experienced workers in that field reflects the fastidious nature of methanogens.

The mechanism by which methanogens reduce carbon dioxide to methane has not yet been fully elucidated. In the process of reducing carbon dioxide to methane, 8 electrons are derived from 4mol of hydrogen and these electrons are used to reduce 1mol of carbon dioxide. Methanogenic bacteria do not possess cytochromes or quinones but have unusual electron carriers, (Wolfe 1979). Coenzyme M and F<sub>420</sub> are among seven new coenzymes and factors isolated from methanogens. Coenzyme M is involved in the methylreductase system whereby carbon dioxide is reduced to

methane and is a methyl carrier in methanogenesis. Coenzyme F<sub>420</sub> is a low-potential electron carrier. The NADP linked hydrogenase system is F<sub>420</sub> dependent. In glycolysis nucleotide cofactors are reduced and these must be reoxidized. Regeneration of NAD<sup>+</sup> can take place in the formation of certain end products such as propionate, valerate, ethanol, lactate and butyrate. Hydrogen producing organisms have another possibility of regenerating NAD<sup>+</sup> by the formation of hydrogen gas. This process is only thermodynamically possible when the partial pressure of hydrogen is kept low. This is achieved by the rapid utilization of hydrogen by methanogens and other hydrogen utilizers, (Prins 1979).



Postgate (1969) reported the synthesis of small amounts of methane by extracts of Desulfovibrio and Clostridium pasteurianum. In contrast to extracts of Methanobacterium in which the carboxyl group of pyruvate is the precursor of methane, in extracts of Desulfovibrio and C. pasteurianum the methyl group is the precursor of methane.

Single dose test meals of pentose monomers, D(+) xylose and L(+) arabinose, have been shown to increase breath methane and hydrogen excretion. The complex polysaccharide, gum arabic, increased methane excretion in the rat. In vitro culture of L(+) arabinose and gum arabic increased hydrogen production with C. histolyticum. Both M.ruminantium and M. barkeri gave increased methane concentrations when incubated with L(+) arabinose. These simple in vitro experiments with a variety of substrates gave confusing results. The reasons for these results are uncertain but indicate that more detailed work with both pure and mixed bacterial cultures is required.

Dietary manipulations may alter the metabolic activity of intestinal bacteria without changing the number or types of bacteria

present, (Finegold and Sutter, 1978). Indeed Smith and Bryant (1979) showed that hydrogen utilizing bacteria such as methanogens alter the products formed by hydrogen producing fermentative bacteria.

Knowledge of bacterial metabolism in the colon is limited. Human methane production may result from the metabolism of a small number of methanogens or the production of small amounts of methane by large numbers of other gastrointestinal organisms such as Clostridia. There may even be two species of methane producing bacteria present in some subjects. It has yet to be demonstrated that differences in methane excretion reflect differences in the number or types of methane producing bacteria. A change in colonic conditions for example, pH or  $PO_2$  may stimulate Clostridia to change their metabolism from mainly hydrogen production to methane production.

#### CONCLUSIONS AND FUTURE AREAS OF RESEARCH

The proportions of methane producing subjects in two healthy populations investigated in Edinburgh were 33% and 70% respectively.

Dietary intakes, faecal components and bowel function, for example stool weight and intestinal transit time were found to be similar in methane producing and non-methane producing subjects. An association exists between the ingestion of the pentose fraction of non-cellulosic polysaccharides and the concentration of breath methane excreted. This association may be the result of a steady metabolic state in the caecum. Ingestion of pentose monomers D(+) xylose and L(+) arabinose increased methane excretion, in methane producing subjects, over 5 hours of study. The lack of gas production following acute complex polysaccharide

administration could be due to a relatively slow metabolic response of colonic bacteria. Production of methane following the acute administration of free pentoses and the absence of methane production after the ingestion of polysaccharide sources may indicate that the release and availability of free pentose monomers from plant polysaccharides may be rate limiting steps in this process.

Patient groups with clinically defined diseases appear to have altered prevalences of methane production compared to control populations. Patients with diarrhoeal states likely to have a rapid colonic transit time had a lower prevalence of methane excretion whereas constipated patients and those with lower abdominal atherosclerosis had a higher prevalence of methane production. It is not clear from the clinical investigations whether methane production predisposes certain diseases or is a consequence of these clinical conditions or indeed whether the absence of methane production is a primary causative factor in certain diseases. In healthy populations there is great variation in the proportion of methane producers and the concentrations excreted. It is unlikely that an individual's methane status could be used as a diagnostic aid. Results of methane status may only be significant in population studies.

Human methane production may be limited by the presence or absence of several factors such as appropriate substrates, symbiotic micro-organisms, inhibitors and methane catabolising micro-organisms. The intestinal transit time and in particular the residence time of contents in the caecum may also influence the colonic environment. These factors may influence the colonic bacterial flora and hence methane production.

The inaccessibility of the human caecum necessitates the use of animal models and in vitro cultures. Caecectomy and the feeding of

an elemental diet to intact rats abolished methanogenesis. Methanogenic bacteria appear to colonise the caecum and require a fibrous residue or matrix for colonisation and as a substrate. Addition of the polysaccharide, gum arabic to the control diet significantly increased methane excretion whereas methane excretion remained absent when gum arabic was added to an elemental diet. Increased methane excretion increased volatile fatty acid concentration and changes in proportions of various volatile fatty acids in the faeces are associated with gum arabic degradation. Changes in caecal environment alter these relationships with gum arabic metabolism.

Small but significant amounts of methane were produced during in vitro culture with three test strains, Clostridium histolyticum, C. perfringens and C. septicum. The simple in vitro experiments with substrates gave confusing results, however, gum arabic and L(+) arabinose increased hydrogen production by C. histolyticum and L(+) arabinose increased methane production by the control methanogens. Human methane production may result from the metabolism of other gastrointestinal organisms such as Clostridia.

Metabolic data on species in pure culture must be interpreted cautiously because they may differ from data obtained with the same species in vivo where many species interact together. However the results from the bacteriological experiments reported in this thesis indicate that more work with both pure and mixed cultures is required. Experiments using  $^{14}\text{C}$  labelled substrates may give information on bacterial metabolism. Studies with mixed organisms in continuous culture may be of value in identifying maintenance requirements of the organisms and the molar growth yields. Methods of refining the techniques required to assess bacterial mass in faeces and colonic contents are required.

Research could also include studies of the enzymatic activities such as polysaccharide degrading activity of bacteria from faeces, colonic contents, defined bacterial mixtures of intestinal species and individual species.

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APPENDIX TO CHAPTER 5

DIAGNOSIS OF THE GENERAL GASTROINTESTINAL PATIENTS. N = 102

Diagnosis	Number of patients
Flatulence from aerophagia	2
Diarrhoea of unknown cause	15
Hiatus hernia with oesophageal stricture	1
Abdominal pains	2
Post vagotomy/gastro-enterostomy diarrhoea	24
Post cholecystectomy diarrhoea	6
Lactase deficiency	9
Diverticular disease	5
Pancreatic carcinoma ? steatorrhoea	1
Coeliac disease	5
Post radiation ileitis	3
Diarrhoea secondary to ileal resection	1
Polya gastrectomy	5
Bacterial colonisation due to achlorhydria	2
Post cholecystojejunostomy diarrhoea	1
Giardia	1
Laxative abuse?	2
Peptic oesophagitis	1
Anorexia nervosa?	1
Rectal ulcer	1
Jejunal diverticulosis	2
Milk sensitivity	1
Duodenal pseudo-obstruction	1
Shigella infection	1
Liver carcinoma/obstruction of caecum	1
Bacterial colonisation	2
Total gastrectomy/gastric lymphoma	1
Ileo-colic tuberculosis/ileal resection	1
Autonomic neuropathy	1
Disordered small intestinal motor activity	1
Diarrhoea, IgA deficient	1
Epigastric pain ? alcohol	1

Steatorrhoea N = 19

Patient	Description	Breath test following a 12hr fast	
		H <sub>2</sub> (μmol/1)	CH <sub>4</sub> (μmol/1)
M.S.	F Polya gastrectomy 24.8mmol/day	< 0.2	-
M.D.	F Post vagotomy diarrhoea, gastroenterostomy 19.6mmol/day	< 0.2	-
V.M.	F ? post vagotomy, fundal plication 26.0mmol/day	< 0.2	-
C.H.	F Polya gastrectomy, truncal vagotomy, adhesions, cholecystectomy, 36.7mmol/day	< 0.2	-
A.B.	M Gastroenterostomy and vagotomy, alcoholic gastritis, 33.3mmol/day	< 0.2	-
H.A.	M Truncal vagotomy and pyloroplasty, diverticular disease 18.8mmol/day	< 0.2	-
G.W.	M Truncal vagotomy 35mmol/day	< 0.2	-
P.D.	M Partial gastrectomy, post vagotomy diarrhoea	< 0.2	-
M.C.	F Gastroenterostomy and vagotomy 34mmol/day	< 0.2	0.27
W.W.	M Gastroenterostomy and vagotomy, 53.3mmol/day	< 0.2	2.79

Patient	Description	Breath test following a 12hr fast	
		H <sub>2</sub> (μmol/1)	CH <sub>4</sub> (μmol/1)
S.D.	M Post vagotomy and gastroenterostomy 43.7mmol/day	< 0.2	0.72
G.S.	M Gastroenterostomy, 19.5mmol/day	< 0.2	2.38
M.M.	F Partial vagotomy, steatorrhoea	< 0.2	-
J.Mc	M 14/4 jejuno ileal bypass for morbid obesity, diarrhoea	< 0.2	0.13
J.H.	M 14/4 jejuno ileal bypass for morbid obesity, diarrhoea	< 0.2	0.81
E.C.	F Small bowel resection, mesenteric ischaemia, cholecystectomy 33.9mmol/day	< 0.2	-
J.Mc	F Post irradiation ileitis and colonisation 26.4mmol/day	< 0.2	-
L.C.	F 14/4 jejuno ileal bypass for morbid obesity	< 0.2	-
R.B.	M Gastro jejunocolic fistula 70mmol/day	< 0.2	-

## Ulcerative colitis N = 40

## Proctocolitis

Patient	Salazopyrin	Breath test H <sub>2</sub> ( $\mu$ mol/l)	CH <sub>4</sub> ( $\mu$ mol/l)	Fasting (F), not fasting (NF)
M.Mc.	+	<0.2	-	F pm
D.M.	+	<0.2	-	NF pm
A.G.	+	0.54	-	NF pm
A.R.	+	<0.2	-	NF pm
Mgt.S	+	0.63	-	NF pm
W.B.	+	<0.2	-	NF pm
M.B.	-	<0.2	-	NF pm
A.F.	-	0.54	-	NF pm
J.H.	-	<0.2	-	NF pm
J.S.	-	0.31	-	NF pm
R.S.	+	0.90	-	NF pm
B.D.	+	<0.2	-	NF pm
W.F.	-	<0.2	-	F pm
M.S.	-	<0.2	-	NF pm
M.T.	-	<0.2	-	NF pm
C.G.	-	2.7	-	NF pm
L.H.	+	0.9	-	NF pm
A.K.	+	0.31	-	NF pm
J.M.	+	<0.2	-	NF pm
L.W.	+	<0.2	-	NF pm
S.S.	-	<0.2	-	NF pm
D.G.	-	<0.2	0.27	NF pm
J.W.	-	<0.2	1.08	F pm
R.R.	+	0.9	0.23	NF pm
M.C.	+	<0.2	0.59	NF pm
W.T.	+	0.31	0.45	NF pm
J.C.	+	<0.2	-	F pm

Left sided colitis

Patient	Sex	Salazopyrin	Breath test		Fasting (F), not fasting (NF)
			H <sub>2</sub> (μmol/l)	CH <sub>4</sub> (μmol/l)	
J.Mc	F	+	<0.2	-	NF pm
W.J.	M	+	0.9	-	NF pm
A.T.	M	+	<0.2	-	NF pm
J.H.	F	-	<0.2	0.2	NF pm
Z.I.	F	+	<0.2	-	NF pm
J.R.	F	+	<0.2	-	F
J.S.	F	+	0.67	-	F
<u>Entire colon</u>					
T.D.	M	+	<0.2	-	NF pm
R.K.	M	+	<0.2	-	NF pm
C.B.	M	+	0.31	-	F
W.W.	M	+	<0.2	-	F
<u>Ileostomy</u>					
J.T.	F	+	<0.2	-	F
<u>Colostomy</u>					
M.W.	F	?	<0.2	-	F

Carcinoma of the colon N = 14Sigmoid colon/rectum

Patient	H <sub>2</sub> (μmol/l)	CH <sub>4</sub> (μmol/l)	
W.F.	<0.2	-	F
E.B.	0.58	0.54	NF am
J.D.	0.27	0.58	NF am
M.R.	<0.2	0.76	NF am
J.W.	<0.2	0.9	NF am
M.C.	<0.2	0.67	NF am
M.I.	<0.2	0.18	NF am
M.S.	<0.2	-	NF am
C.T.	<0.2	-	NF am
M.Mc.	<0.2	-	NF am

Caecum/ascending colon

F.C.	F	<0.2	0.18	NF am
G.F.	M	<0.2	-	NF am
K.B.	M	<0.2	-	NF am
D.Mc.	M	<0.2	-	F

(also Crohn's)

Pneumatoxis cystoides intestinalis N = 9

Patient	Description	Breath test following a 12hr fast	
		H <sub>2</sub> ( $\mu$ mol/l)	CH <sub>4</sub> ( $\mu$ mol/l)
J.A.	descending colon (in remission)	0.31	1.53
R.G.	total colon	1.44	-
G.S.	mainly small intestine	1.48	-
J.S.	colonic	2.97	-
W.P.	descending colon	4.14	-
I.S.	descending colon	1.17	-
C.R.	sigmoid colon, resected	3.33	-
N.A.	descending colon	1.39	-
K.P.	descending colon	2.20	-

Crohn's disease N = 39

Colonic only

Patient	H <sub>2</sub> ( $\mu\text{mo l/l}$ )	CH <sub>4</sub> ( $\mu\text{mo l/l}$ )	
J.Mc.	1.8	-	F
A.M.	<0.2	-	F
M.Mc.	<0.2	-	F
M.S.	<0.2	-	F
J.B.	<0.2	1.03	NF pm

Ileo-caecal resection

D.L.	M	1.3	-	NF pm
D.Mc.	F	<0.2	-	F
W.R.	M	<0.2	-	NF pm
A.M.	M	<0.2	-	F
A.B.	M	<0.2	-	F
D.B.	F	<0.2	-	NF pm
P.B.	M	<0.2	-	NF pm
J.G.	F	0.9	-	NF pm
M.H.	F	<0.2	-	F
W.A.	F	<0.2	-	F
M.F.	F	<0.2	-	F
C.Mc.	M	<0.2	-	F
C.R.	M	0.40	-	NF pm
A.S.	F	0.76	-	NF pm
J.P.	M	<0.2	-	F
L.D.	M	<0.2	-	NF pm
A.W.	M	<0.2	-	NF am
G.H.	F	<0.2	0.27	NF pm

ileal disease, no resection

Patient		H <sub>2</sub> (μmol/l)	CH <sub>4</sub> (μmol/l)	
I.S.	F	1.84	-	F
J.C.	F	<0.2	-	NF pm
B.G.	F	<0.2	-	NF pm
H.D.	M	<0.2	-	NF pm
C.P.	F	0.67	-	NF pm
M.F.	F	0.63	-	NF pm
A.W.	F	0.23	0.36	NF pm
D.C.	M	<0.2	0.27	F

small intestinal only

E.W.	F	0.9	-	NF pm
J.R.	M	1.8	0.23	NF pm
K.C.	M	<0.2	-	F
N.S.	M	1.35	-	F
S.F.	M	<0.2	-	NF pm
M.Mc.	F	<0.2	-	F
M.F.	F	<0.2	-	F

Irritable bowel syndrome N = 42

Patient		Breath test following a 12hr fast		Patient		$H_2$ ( $\mu\text{mol}/1$ )	$\text{CH}_4$ ( $\mu\text{mol}/1$ )
		$H_2$ ( $\mu\text{mol}/1$ )	$\text{CH}_4$ ( $\mu\text{mol}/1$ )				
S.B.	M	<0.2	2.25	I.P.	F	<0.2	1.35
B.K.	F	0.9	0.18	M.B.	F	<0.2	0.23
M.T.	F	0.23	0.67	E.S.	F	<0.2	0.45
A.W.	M	<0.2	0.45	S.W.	F	<0.2	0.67
E.C.	F	<0.2	0.27	P.S.	M	<0.2	0.45
J.B.	F	<0.2	0.23	J.S.	F	<0.2	0.23
W.Mc.	M	<0.2	0.67	F.R.	F	<0.2	0.27
S.W.	F	<0.2	0.31	M.H.	F	<0.2	0.72
P.H.	F	<0.2	0.9	J.H.	F	<0.2	-
J.W.	F	<0.2	-	B.A.	F	<0.2	-
G.P.	F	<0.2	-	M.K.	F	<0.2	-
M.G.	F	<0.2	-	I.E.	F	<0.2	-
J.B.	F	<0.2	-	S.P.	F	<0.2	-
S.U.	F	<0.2	-	M.T.	F	<0.2	-
G.T.	M	<0.2	-	J.B.	F	<0.2	-
E.H.	M	<0.2	-	J.S.	F	<0.2	-
T.W.	M	<0.2	-	D.H.	F	<0.2	-
J.S.	M	<0.2	-	S.G.	F	<0.2	-
R.B.	M	<0.2	-	R.C.	M	<0.2	-
G.W.	M	<0.2	-	C.M.	F	0.27	-
W.R.	M	0.23	-	R.B.	M	0.36	-

APPENDIX 2

DESCRIPTION OF CHEMICALS USED

<u>Name of chemical</u>	<u>Source</u>	<u>Purity (grade)</u>
Acetic acid glacial	BDH Chemicals Ltd, Poole, England	Anala R
D arabinose	BDH	Biochemical
L arabinose	BDH	Biochemical
Bran	Chancelot Mills, Edinburgh	Commercial coarse
n-butyric acid	Sigma Chemical Company	
Cellulose	Vitamins Inc. Chicago, USA	Solka Flocc SW2030
Flexical elemental diet	Mead Johnson Laboratories, Division of Bristol-Myers Co. Ltd., Slough, England	
Galactose	BDH	Biochemical
D glucose	Evans Medical Ltd. Speke, England	Biochemical
Gum acacia	Rountree MacIntosh Ltd, The Cocoa Works, York	
Hydrogen gas	BOC	

<u>Name of chemical</u>	<u>Source</u>	<u>Purity (grade)</u>
Hydrogen and methane standards	Rank Precision Ind. Ltd. Kent, England	In N <sub>2</sub>
isobutyric acid	Sigma	Grade 1
isovaleric acid	Sigma	
Lactose	Evans Medical Ltd	Biochemical
Methane	Air Products Ltd	99%
$\beta$ methylvaleric acid	Chromatography Services Ltd. Hoylake, Merseyside, England	
Molecular Sieve 5A	Phase Separation Ltd. Flintshire, England	
Nitrogen	BOC	OFN
Oxoid breeders diet	Oxoid Ltd. Wade Road., Basingstoke, Hampshire England	
Propionic acid	Sigma	
Raffinose	BDH	Biochemical
Rhamnose	BDH	Biochemical
Silica gel	BDH	Biochemical
Soda lime	Medical and Industrial Equipment Ltd. London	

<u>Name of chemical</u>	<u>Source</u>	<u>Purity (grade)</u>
Spratt's small animal diet	Spratt's Patent Ltd. Central House, Cambridge Rd. Barking, Essex, England	
n-valeric acid	Sigma	Sigma grade
Xylan (from Larchwood)	Sigma	
D xylose	BDH	Biochemical
L xylose	Sigma	

## PUBLICATIONS

1. The influence of pentose on breath methane excretion  
Proc. Nutr. Soc., 1981, 40, 74A.
2. Gum arabic metabolism in the rat colon  
Proc. Nutr. Soc., 1981, 40, 73A.
3. The influence of pentose on breath methane  
Am. J. Clin. Nutr., 1981, in press.
4. Methane and hydrogen production by anaerobic bacteria of the  
human intestine  
J. Med. Microbiol., 1981, submitted.

**The influence of pentose on breath methane excretion.** By LINDA F. MCKAY, W. G. BRYDON, M. A. EASTWOOD and J. H. SMITH, *Wolfson Gastrointestinal Laboratory, University Department of Medicine, Western General Hospital, Edinburgh*

Methane is produced in man by anaerobic bacterial metabolism in the colon and is excreted in flatus and expired breath. Approximately one-third of the adult population excrete methane in the breath (Bond *et al.* 1971).

In this investigation the incidence of methane production in fifty-six healthy subjects aged from 16 to 79 years has been studied in relation to various components of their diet. The mean daily dietary intakes were ( $\pm$ SD)  $68 \pm 19$  g protein,  $99 \pm 32$  g fat,  $226 \pm 83$  g carbohydrate,  $8.9 \pm 2.9$  MJ energy and  $13 \pm 4.9$  g fibre. Of the subjects, thirty-four produced methane in concentrations which ranged from 0.09 to 1.49  $\mu$ mol/l above room concentrations (mean 0.7  $\mu$ mol/l). Breath methane concentrations in methane producers significantly correlated with intakes of the pentose fraction of non-cellulosic polysaccharide ( $r$  0.44,  $P < 0.01$ ) and lignin ( $r$  0.40,  $P < 0.05$ ). The intake of lignin and pentose were correlated ( $r$  0.82,  $P < 0.001$ ). Subsequently, twelve methane producers aged from 28 to 50 years were given test meals containing 5 g pentose as 510 g cooked fruit and vegetables; 385 g orange; 22.1 g bran. Other sources of pentoses 10 g xylan; 25 g D+xylose; 25 g L-xylose; 20 g L+arabinose or 20 g D-arabinose were given to fasting subjects. Breath methane concentrations were measured before the test meal and every half hour for 5 h. Measurements were also taken during a control period of fasting.

The acute administration of complex polysaccharide sources rich in pentose had no significant effect on breath methane production over 5 h. The administration of D+xylose and L+arabinose led to a significant increase ( $P < 0.01$ ;  $P < 0.025$  respectively, Wilcoxon Signed Rank Test) in methane excretion both at 90 to 120 min compared to base line and at 210 to 240 min compared to the measurement 1 h previously. Both L-xylose and D-arabinose caused diarrhoea.

The first peak of the biphasic methane response after ingestion of pentose monomers is probably caused by the fermentation of the unabsorbed fraction of pentose passing into the caecum.

The association between breath methane concentration and the ingestion of pentose containing fibre is the result of a steady metabolic state in the caecum. The lack of gas production following acute complex polysaccharide administration could be due to a relatively slow metabolic response of bacteria. The release and availability of free pentose monomers from plant polysaccharides may be rate limiting steps in this process.

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Bond, J. H., Engel, R. R. & Levitt, M. D. (1971). *J. exp. Med.* 133, 572.

**Gum arabic metabolism in the rat colon.** By A. H. McLEAN ROSS, L. F. MCKAY, A. BUSUTTIL, D. M. W. ANDERSON, W. G. BRYDON and M. A. EASTWOOD, *Gastrointestinal Unit and Pathology Department, Western General Hospital, and Department of Chemistry, University of Edinburgh*

Fibre is a complex heterogeneous material increasingly used in the management of colorectal and other diseases (Heaton, 1978). It is now appreciated that cereals, bran, and vegetable fibre behave differently along the gastrointestinal tract (Stephens & Cummings, 1980). Indirect evidence suggests the caecum is a major site for metabolism of certain fibres.

We have studied the metabolism of a chemically defined, readily identifiable polysaccharide, gum arabic (GA) in the rat intestine. GA is a water soluble polysaccharide (molecular weight approximately 850 000) which contains rhamnose, arabinose, glucuronic acid and galactose. It is used as an ingredient in some foodstuffs such as confectionery industry.

Incubation experiments using human pancreatic and gastric juices demonstrate no degradation of the molecule after 48 h.

Wistar rats (3-month-old) were fed on gum arabic, incorporated in pellets of reconstituted oxoid breeders diet, at doses of 0–200 g/kg, for a period of 4 weeks. Other rats were fed on a complete elemental diet (residue free) containing gum arabic (220 g/kg dry weight) in jelly form administered over the same time period.

Intestinal contents were examined for precipitable GA by the addition of acidified ethanol. GA was found from stomach to small intestine but not in the caecum, colon, or rectum. Caecal excision and reconstitution of intestinal continuity resulted in GA recovery from stomach to rectum.

Excreted methane, hydrogen, and intestinal volatile fatty acids (VFA) were measured as indicators of bacterial activity in the caecum and colon.

Methane excretion, a measure of caecal bacterial metabolism, increased significantly ( $P < 0.001$ ) on the GA pellet diet. Hydrogen concentrations remained unaltered. Methanogenesis ceased on elemental diets alone or gum supplemented and following caecal excision.

Faecal VFA concentrations increased in a linear fashion with increasing doses of GA ( $r = 0.860$ ). Acetate concentrations increased ( $r = 0.972$ ) and butyrate concentrations decreased ( $r = 0.888$ ) with increasing GA dosage. Significant decreases in concentrations of VFAs were found from caecum to left colon ( $P < 0.005$ ) and from (L) colon to faeces ( $P < 0.05$ ) on control diet but not on GA pellet diet. It can be concluded that (1) GA degradation occurs in the caecum and is associated with increased methane excretion, increased VFA concentration and changes in the proportions of various VFAs in the faeces. (2) Changes in caecal environment alter these relationships with GA metabolism.

Heaton, K. W. (ed.) (1978). *Dietary fibre. Current developments of importance to health.* J. B. Libby & Co. Ltd.

Stephens, A. M. & Cummings, J. H. (1980). *Nature, Lond.* 284, 5753.