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Methane excretion in man – a study of breath, flatus, and faeces

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SUMMARY In this paper aspects of the variability of methane producing status have been examined, and a survey of breath methane excretion in various clinical and control populations is reported. Prevalences of methane excretion were 54% in healthy controls, 53% in non-gastrointestinal patients and 32% in gastrointestinal patients. Patients with Crohn's disease, ulcerative colitis, and pneumatosis cystoides intestinalis had significantly lower prevalences of methane excretion (13%, 15%, and 11% respectively). Faecal constituents and *in vitro* incubation analysis were similar in breath methane excretors and non-excretors. Several patients did not excrete methane in the breath although methane was present in colonic gas. The results indicate that different gastrointestinal patient groups have different prevalences of breath methane excretion and that all healthy subjects may produce methane but only when the production reaches a threshold does it appear in the breath.

Methane and hydrogen are produced during anaerobic bacterial activity in the large intestine and are excreted in flatus and expired breath. Human intestinal tract fermentation produces hydrogen, but methane is neither a universal constituent of colonic gas nor of expired breath.[1 2]

Reports of the proportion of healthy populations which excrete methane in the breath range from 33–60%.[3–5] Methane excretion is not significantly affected by age and sex,[3] although diet,[5] bacterial flora,[6 7] ethnic origin,[4] intestinal transit time,[8] and a familial component may be involved.[3]

Methanogenic bacteria utilise hydrogen, carbon dioxide, and to some extent formate.[9] They are strict anaerobes[10] and colonise the human large intestine and faeces.[6] In the human it is still not clear whether methane results from the metabolism of a few methanogens or a large number of gut organisms such as bacteroides and Clostridia.[7]

In this paper a survey of breath methane excretion in various clinical and control populations is reported. Measurements have been made of faecal constituents, intestinal transit time, and the ability of faecal microorganisms to produce methane *in vitro*, all of which could influence colonic bacterial activity and methane excretion status.

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Methods

SUBJECTS

Group 1

The control population consisted of 142 hospital staff, students, and local volunteers (74 men, 68 women) aged 16 to 79 (mean 35) years. Local volunteers were randomly selected by their family doctor, and all subjects were considered to be free of gastrointestinal disease.

Group 2

The clinical population consisted of 245 patients attending the Gastrointestinal Unit, Western General Hospital, 104 men and 141 women aged 16 to 73 (mean 49) years. The clinical diagnoses in this group were as follows: unresected colonic carcinoma (20), Crohn's disease (40), ulcerative colitis (40), irritable bowel syndrome (42), pneumatosis cystoides intestinalis (nine) and a group with diarrhoea (94) of various aetiologies outlined in Table 1. Criteria used to establish a clinical diagnosis included a clinical history and investigations as appropriate – for example, endoscopy, barium meal examination, pancreatic function tests, enteric biopsies, and haematological and

Table 1 *Details of non-specific diarrhoeal patients*

Diagnosis	*Patients (no)*	*Diagnosis*	*Patients (no)*
Post vagotomy/gastro-enterosotomy	24	Giardiasis	1
Diarrhoea of unknown aetiology	15	Laxative abuse	2
Post cholecystectomy diarrhoea	6	Rectal ulcer	1
Lactase deficiency	9	Jejunal diverticulosis	2
Diverticular disease	5	Milk sensitivity	1
Coeliac disease	5	Duodenal pseudo-obstruction	1
Polya gastrectomy	5	Shigella infection	1
Post radiation ileitis	3	Total gastrectomy/gastric lymphoma	1
Small intestinal colonisation	5	Ileo-colic tuberculosis/ileal resection	1
Pancreatic carcinoma	1	Autonomic neuropathy	1
Diarrhoea secondary to ileal resection	1	Disordered small intestinal motor activity	1
Post cholecystojejunosotomy diarrhoea	1	IgA deficient diarrhoea	1

biochemical analyses. The diagnosis of irritable bowel was given to patients with lower abdominal pain and diarrhoea, the pain being relieved by the passage of stool, often of a pelletty nature and accompanied by mucus. Sigmoidoscopy and barium enema were normal. Diarrhoea of unknown origin was the frequent passage of unformed stool with no identifiable cause and with none of the features of the irritable bowel syndrome.

Group 3

This clinical group consisted of 64 patients, 35 men, 29 women, aged 17 to 86 (mean 48) years, age matched patients in the general hospital wards who were considered free of gastrointestinal disease as assessed by routine clinical history and examination.

Subjects and patients taking antibiotics or laxatives or who had received enemas or intestinal washouts within the previous month[11] or who were on elemental diets as part of their preoperative bowel preparation, were excluded from this study. Several patients with inflammatory bowel disease were taking salazopyrine (ulcerative colitis, n=26 and Crohn's disease n=24). This is discussed further in the results.

BREATH ANALYSIS

All subjects provided two (non-fasting) end-expiratory breath samples, 40 ml each, using a modified Haldane-Priestley sampling tube.[12] Background room air was also sampled. No subjects smoked before breath sampling.[13]

Methane concentrations were determined by gas chromatography[14] (Pye Series 104; Pye, Cambridge, England) using molecular sieve (3A 60/85 mesh) packed glass columns and a katharometer detector. A range of attenuations were used and calibration curves were constructed using serial dilutions of pure methane (BDH Chemicals Ltd, Poole, Dorset, England). This method detects gas concentrations of 0·09 μmol/l and above. The sensitivity for 1 μmol/l methane is 10% of full scale deflection of recorder and the analytical reproducibility, coefficient of variation less than 3% for n=20. Breath methane concentrations were taken as the difference between the mean of the two breath samples and room air concentrations and methane producers were defined as those subjects producing at least 0·09 μmol/l of methane above room air concentration. This criterion was based on the sensitivity and reproducibility of the method used and the results of previous investigations.[5 15] Methane production is not affected by the time of food ingestion, or fasting.[3 15 16]

RECTAL GAS ANALYSIS

In 20 subjects from group 2, Crohn's disease (five), ulcerative colitis (eight), irritable bowel syndrome (six), colonic carcinoma (one), gas was also sampled in the rectosigmoid area between 7 and 10 cm at sigmoidoscopy by passing a rubber tube attached to a syringe, through a sigmoidoscope. The sigmoidoscope was passed up to 7 to 10 cm without pumping air. The end of the sigmoidoscope was sealed in such a manner that the tube could be inserted without room air contaminating the colonic gas. A further check on the purity of this gas sample was made by analysing the hydrogen content, using the same method as for methane analysis.[14] All gas samples contained a minimum of 11·5 μmol/l hydrogen (room air may contain up to 0·09 μmol/l), indicating that colonic gas, rather than room air contaminating the sigmoidoscope, was being measured.

INTESTINAL TRANSIT MEASUREMENTS AND FAECES ANALYSIS

Seventy one subjects from group 1 (41 men, 30 women) collected stools for intestinal transit measurements. Stools were collected individually and stored at −20°C. Intestinal transit time was

measured using barium impregnated markers, as described by Hinton *et al.*[17] Markers in the frozen stool were identified under a fluoroscope. Transit time was assessed as the time taken for the passage of 80% of the swallowed markers.

Five day faecal collections were pooled, thawed, homogenised, and an aliquot of known weight freeze dried. Dried faeces was analysed for bile acids,[18] fat,[19] and electrolytes which were measured by flame photometry and atomic absorption spectrophotometry after charring with nitric acid.

IN VITRO FAECAL INCUBATION

Sixteen subjects from group 1 provided freshly passed stool. One gram of fresh stool was homogenised with 10 ml 0·2 M phosphate buffered saline, (PBS) pH 7·0. One millilitre of homogenate was added to 10 ml brain heart infusion broth – that is, 10^{-3} g faeces/ml brain heart infusion) in screw capped bottles fitted with perforated caps with rubber seals to facilitate sampling of head space gas. Inocula consisting of 10^{-2} g faeces/ml phosphate buffered saline were also prepared. All incubations were set up in duplicate and left at 37°C for 48 hours after which time head space gas was analysed for methane. Preliminary studies showed that 10^{-3} g faeces/ml brain heart infusion yielded significant concentrations of methane gas from slow growing methanogens after 48 hours with the substrate rich broth. With a weak buffer (PBS) and no additional substrate the dilution of 10^{-2} g faeces was required to yield measurable concentrations of methane gas.[7]

Results were analysed using mean, standard deviation, χ^2 test and linear regression analysis.

Results

BREATH ANALYSIS

The breath methane prevalence and concentrations in control and patient groups are given in Table 2. For the control population (group 1) there was no significant difference between the prevalence of methane excretion in men and women 58% and 50% respectively, and breath methane concentration was not influenced by age (r=0·09). The overall prevalence of methane production in patients with gastrointestinal disease (group 2) is 32% and in non-gastrointestinal patients (group 3) is 53%. Within the gastrointestinal patient group there were differences in the prevalence of methane production. Patients with Crohn's disease, ulcerative colitis, and pneumatosis cystoides intestinalis had a significantly decreased prevalence of methane excretion compared with non-gastrointestinal patients ($p<0·001$, $p<0·001$, $p<0·025$ respectively χ^2 test). Patients with colonic carcinoma and diarrhoea who were methane excretors produced significantly more methane ($p<0·005$, $p<0·001$ respectively) than the non-gastrointestinal patients.

The prevalence and concentration of methane in patients with Crohn's disease, and ulcerative colitis were independent of disease distribution (Table 3). Methane was excreted in the breath of three of the 24 Crohn's patients taking salazopyrin and three patients of the 26 patients with ulcerative colitis taking salazopyrin. These are proportionally the same as the overall group, 13% and 12% respectively.

RECTAL GAS ANALYSIS

Results of breath and colonic gas analyses are shown in Table 4. The presence or absence of methane in breath and colonic gas was concordant in 15 patients. Patients excreting detectable concentrations of methane in the breath (n=3) excreted high concentrations in the colonic gas (>30 μmol/l). Methane was not detected in either the breath or the colonic gas from all ulcerative colitis patients and two patients with Crohn's disease. The mean methane concentration detected in the colonic gas of three patients with Crohn's disease and two with irritable bowel syndrome, who did not excrete methane in the breath, was 1·48 μmol/l.

Table 2 *Incidence and concentrations of breath methane in control and patient groups*

Group	*Diagnosis (no)*	*Methane excretors (%)*	*Breath methane (μmol/l)*		
			Range	*Mean*	*SD*
1	Controls (142)	54	0·09–2·43	0·77	0·52
2	Colonic carcinoma (20)	50	0·18–3·42	0·99	0·90
2	Crohn's disease (40)	13	0·23–1·03	0·43	0·33
2	Ulcerative colitis (40)	15	0·2–1·08	0·47	0·33
2	Pneumatosis cystoides intestinalis (9)	11	1·5 (one patient)		
2	Irritable bowel syndrome (42)	40	0·28–2·25	0·60	0·52
2	Diarrhoea (94)	42	0·09–5·40	1·12	1·08
3	Non-GI disease (64)	53	0·09–1·84	0·54	0·45

Table 3 *Details of Crohn's disease (n=40) and ulcerative colitis (n=40) disease distribution and methane excretion*

Disease distribution	*Patients (no)*	*Methane excretors (no)*
Crohn's disease		
Colonic	7	1
Ileocaecal resection	18	1
Small intestine only, no resection	15	3
Ulcerative colitis		
Proctocolitis	29	5
Left-sided colitis	7	1
Entire colon	4	0

Table 5 *Faecal constituents and transit time for group 1 subjects (n=71)*

	Methane excretors (n=42)		*Non-excretors (n=29)*	
	Mean	*SD*	*Mean*	*SD*
Wet weight g/day	101	39	128	44
Dry weight g/day	27	8	33	10
Fat mmol/day	11·4	5·2	13·3	7·1
Total bile acids mmol/day	0·70	0·29	0·81	0·30
Total electrolytes mmol/day*	35·5	16·4	39·3	18·2
Total neutral sterols mmol/day	1·82	0·94	1·79	0·61
Transit time/hours	72	30	62	28

* Sum of sodium, potassium, calcium and magnesium.

INTESTINAL TRANSIT MEASUREMENTS AND FAECAL ANALYSIS

Table 5 has details of faecal constituents and transit time for methane and non-excretors in 71 subjects from group 1. There were no significant differences between methane and non-methane excretors, and no associations between the concentration of methane excreted and any faecal constituent.

IN VITRO FAECAL INCUBATIONS

There were no significant differences in headspace gas % methane between inocula from breath methane excretors (n=10) range 0·012–0·015%, and non-excretors (n=6) range 0·012–0·013% in phosphate buffered saline alone. In the presence of brain heart infusion broth, inocula from methane excretors yielded significantly more methane, range 2·37–5·97%, than non-excretors, range 0·95–1·78% ($p<0·001$).

Discussion

The objective of this study was to investigate and compare the prevalence of methane excretion in clinical and non-clinical groups. Reasons for the diversity in methane excretion have been examined in studies of colonic function measurements and faecal incubations from methane and non-methane producers.

Table 4 *The occurrence of methane in breath and colonic gas*

		Breath µmol/l	*Colonic gas µmol/l*
Colonic carcinoma	n=1	+ (0·45)	+ (>30)
Crohn's disease	n=2	—	—
	n=3	—	+ (<4·0)
Ulcerative colitis	n=8	—	—
Irritable bowel syndrome	n=2	—	—
	n=2	—	+ (<4·0)
	n=2	+ (0·99, 2·97)	+ (>30)

All human intestinal tract fermentations produce hydrogen but methane is not a universal constituent of colonic gas or expired air. Methanogenic bacteria may colonise the lumen or the mucosal epithelium of the colon. A lack of methanogenic activity may reflect rapid passage of intestinal contents, an altered epithelial condition or differences in bacterial activity and physical conditions in the colon.

There are wide variations reported in the proportion of methane excreting subjects in different healthy adult populations ranging from 33[3]–58%[4] in North America, 40[20]–61%[5] in Britain, and 75—80% in Nigeria.[21] The methane excreting status of an individual has been shown to remain stable over periods up to three years.[3]

The similarity in methane production after faecal fermentation both in healthy breath methane excretors and non-excretors may indicate that methane is produced by all subjects but in varying concentrations, and that only when the production reaches a threshold level does methane appear in the breath. Previous *in vitro* fermentation studies have shown methane production to be stimulated by added amino acids[22] and glycoproteins.[23] Although all faecal incubations yielded methane, incubations with brain heart infusion broth, a complex growth medium produced significantly more methane using stool from breath methane excretors. This may reflect differences in bacterial populations, increased activity of methanogens or greater numbers of methanogens in stool from breath methane excretors. Differences in methane absorption from the colon into the bloodstream and transport to the lungs, however, may also exist.

The prevalence of methane excretion in small groups of patients with various clinical conditions

including faecal impaction, liver disease, and in patients receiving intravenous alimentation has been studied.[24] There were no significant differences in methane concentrations between any of those clinical patients, however, the possible effect of antibiotics on colonic bacteria was not considered. Haines *et al*[20] found that 80% of patients with large bowel carcinoma (n=30) excreted methane compared with 40% of non-gastrointestinal patients, whereas other workers,[11] showed that only 42% of patients with unresected large bowel carcinoma (n=55) excreted breath methane.

The incidence of methane excretion in our survey was not associated with age in either the non-clinical or clinical populations. The only subgroups with significantly different prevalences of methane excretion were patients with pneumatosis cystoides intestinalis (11%), Crohn's disease (13%) and ulcerative colitis (15%).

These conditions are chronic diarrhoeal states with rapid colonic transit times. Although transit measurements were not made in those patients variations within the normal range (68·4–29·5 hours) did not influence methane excretion status. Also, patients with diarrhoea from other causes do not have a significantly different prevalence of methane excretion (42%) compared with non-gastrointestinal patients (53%).

The absence of methane excretion with inflammatory bowel disease and pneumatosis cystoides intestinalis may result from an altered epithelial mucosa. Analysis of colonic gas showed a lack of methane production in patients with inflammatory bowel disease and also several patients (n=5) with no detectable methane in the breath had low concentrations in the colonic gas. Patients with inflammatory bowel disease may lack the capacity for significant methane production or excretion, which may result from differences in relative oxygen tension, blood flow or membrane condition and permeability. A group of patients with conditions associated with possible reduced colonic blood flow have been shown to have an increased incidence of breath methane.[25]

Increased concentrations of breath methane were observed in methane excretors with colonic carcinoma and diarrhoea. These patients may have a greater population of methanogenic bacteria, fewer methane utilising bacteria which oxidise methane yielding carbon dioxide or altered colonic mucosal absorption. These observations present an intriguing problem meriting further study.

The variation in the percentage of individuals excreting methane in normal and clinical populations raises the question as to why individuals produce and excrete methane. It has been suggested that intestinal transit time is an important factor in producing methane,[8] however, from this study and previous work[5] it seems unlikely that transit time significantly affects methane production. It is possible that methane producing bacteria colonise the mucosa of the distal intestine where there is a complex microbial ecology.[26] This will affect their response to different intestinal diseases. Several inflammatory colonic diseases, however, significantly reduce the prevalence of methane production. We tentatively conclude that methane production in the colon is the norm and that a number of individuals do not excrete methane in the breath possibly as insufficiently high concentrations are generated in the colon.

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