

Alkyl halides, super hydrogen production and the pathogenesis of pneumatosis cystoides coli

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Abstract

Background and aims—The colons of patients with pneumatosis cystoides coli produce excessive H₂. Exposure to alkyl halides could explain this. Six consecutive patients who had pneumatosis cystoides coli while taking chloral hydrate (1–5+ g/day) are reported. Patients 2 and 3 were investigated after they had ceased chloral hydrate treatment. One produced methane, the other did not. (Pneumatosis cystoides coli patients are non-methanogenic according to the literature.) Both had overnight fasting breath H₂ of less than 10 ppm. A literature review disclosed just one patient who was using chloral at the time of diagnosed pneumatosis cystoides coli, but an epidemic of the disease in workers exposed to trichloroethylene.

Methods—(i) In vitro experiments with human faeces: chloral or closely related alkyl halides were added to anaerobic faecal cultures derived from four methane-producing and three non-methanogenic human subjects. H₂ and CH₄ gases were measured. (ii) In vivo animal experiment: chloral hydrate was added to drinking water of four Wistar rats, and faecal H₂ compared with control rats.

Results—Alkyl halides increased H₂ up to 900 times in methanogenic and 10 times in non-methanogenic faecal cultures. The K_i of chloral was 0.2 mM. Methanogenesis was inhibited in concert with the increase in net H₂. In the rat experiment, chloral hydrate increased H₂ 10 times, but did not cause pneumatosis.

Conclusions—Chloral and trichloroethylene are alkyl halides chemically similar to chloroform, a potent inhibitor of H₂ consumption by methanogens and acetogens. These bacteria are the most important H₂-consuming species in the colon. It is postulated that exposure to these alkyl halides increases net H₂ production, which sets the scene for “counterperfusion supersaturation” and the formation of gas cysts. In recent times, very low prescribing rates for chloral have caused primary pneumatosis cystoides to become extremely rare. As with primary pneumatosis, secondary pneumatosis cystoides, which occurs if there is small bowel bacterial overgrowth distal to a proximally located gut obstruction, is predicted by counterperfusion supersaturation. “Inherent unsaturation” due to metabolism of O₂ is a safety factor, which could explain

why gas bubbles do not form more often in tissue with high H₂ tension.

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Pneumatosis cystoides intestinalis is a rare disorder which is characterised pathologically by the presence of gas-filled cysts in the walls and mesentery of the small or large intestine.^{1–5} The cysts are found near blood vessels mostly on the mesenteric border.^{3 5 6} Pneumatosis cystoides intestinalis affecting the small intestine has commonly been reported in association with organic intestinal obstruction—for example, pyloric stenosis^{2 7} and Crohn's stricture³—or functional pseudo-obstruction.^{8–10} Acute pneumatosis cystoides intestinalis has also been described in neonates with necrotising enterocolitis⁵ and in immunocompromised children¹¹ and adults.^{12 13} Invasion of the gut wall by gas-forming bacteria undoubtedly occurs in these settings of overwhelming gut infection or severely compromised immune function.

On the other hand, pneumatosis cystoides intestinalis that is confined to the colon, pneumatosis cystoides coli (PCC), has been reported mainly in an older population.^{4 9 14–19} There is no recognised predisposing cause—that is, PCC has been characterised as a primary idiopathic condition—and, in particular, bacteria are not seen in the gut wall or mesentery with PCC.¹⁸ Association with chronic pulmonary disease⁶ has led to speculation about a possible relationship to changes in blood gas tensions.⁹

An account of the pathogenesis of PCC must explain (a) why the cysts form ab initio, (b) why the cysts are then maintained in the colonic wall and mesentery, (c) why the cysts contain H₂ gas^{9 14 15} but not CH₄,^{1 9} (d) why PCC patients are super H₂ producers,^{4 14 16 17} and (e) the epidemiology of the disease.

There is theoretical and experimental evidence that gas cysts can form by counterperfusion supersaturation of metabolically inert gases,²⁰ which would explain (a), (b) and (c). Counterperfusion supersaturation of gases is analogous to a form of decompression sickness in deep sea divers but where there is normal atmospheric pressure.²⁰ It would occur in the colon only if H₂ gas pressure in the lumen were so high as to be equal and opposite to N₂ gas pressure in the blood. This condition does not occur in normal humans, where metabolism of colonic bacteria produces H₂, but luminal H₂

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tension remains less than N_2 tension in the colonic lumen.²¹ This is because H_2 tension is reduced by a combination of H_2 consumption by bacteria,²²⁻²⁴ H_2 excretion in flatus or breath,^{25, 26} and N_2 , which by entering the lumen, dilutes H_2 tension. N_2 may enter the gastrointestinal lumen via diffusion from blood and tissue²⁰ or via swallowed air.²⁷ On the other hand, super H_2 production by colonic bacteria, which occurs in patients with PCC, provides the condition for H_2 tension in the colonic lumen to approach the level of N_2 tension in blood. This permits counterperfusion supersaturation,²⁰ which can occur provided that the inherent unsaturation of tissue due to metabolism of O_2 is significantly less than the peak supersaturation due to opposing gradients of H_2 and N_2 .²⁰ It remains to be explained why patients with PCC are super H_2 producers^{4, 14, 16, 17} and the epidemiology of this rare disorder.

This study comprises clinical and experimental data, which demonstrate why many patients with PCC are super H_2 producers. Six consecutive cases of PCC are reported. Three were investigated by the author. All six were prescribed chloral hydrate, 1-5+ g/day. Chloral, which is probably the oldest synthetic chemical used in hypnotic drug therapy, is rapidly metabolised to trichloroethanol, the metabolite responsible for its hypnotic action.²⁸ The plasma half-life of trichloroethanol in humans is eight hours.²⁹ Other metabolites of chloral hydrate include trichloroacetic acid and trichloroethanoglucuronide, which is excreted by the liver.³⁰ These compounds are alkyl halides like trichloroethylene and chloroform. It is relevant that trichloroethylene, which is used in the watch-making and camera industry, was recently associated with an epidemic of PCC in Japan.³¹ Trichloroethylene is also metabolised by the liver to trichloroethanol and trichloroacetic acid.

The effect of these alkyl halides on net H_2 production was examined in vitro in human faecal cultures and in vivo in an animal model. The data predict why humans exposed to alkyl halides would become super H_2 producers, and are discussed in relation to the pathogenesis of idiopathic PCC and small intestinal pneumatosis cystoides.

Patients and Methods

PATIENT 1

An 83 year old female nursing home patient presented to the author with confusion and dehydration in 1989. She was incontinent of faeces which were noted to be blood-stained. She was on β -blocker therapy for hypertension, haloperidol and chloral hydrate 1-2 g nocte. Flexible sigmoidoscopy showed characteristic PCC cysts in the sigmoid colon, which were about 0.5-1 cm in diameter and which made a hissing sound when punctured with a biopsy forceps. Her faeces were weakly methanogenic (see below). Fasting H_2 breath, which is abnormally high in PCC,^{4, 14, 16, 17} was 34 ppm. Breath CH_4 was not measured. The patient received no specific treatment and died shortly after.

PATIENT 2

This female patient (date of birth 15 October 1928) had been well until 1972, when she suffered the unexpected death of a family member. She was prescribed diazepam and chloral hydrate at the time of the bereavement to help her sleep. In 1974, the patient was referred to a gastroenterologist; she had a six month history of altered bowel habit, mucus, and occasional blood. Plain abdominal radiographs and barium enema investigation showed PCC involving the sigmoid and descending colon. She received antibiotics and oxygen therapy between 1975 and 1982 but treatment responses were not sustained. A progress barium study in 1986 showed extensive PCC.

In 1994, patient 2 was seen by the author. Directed questioning revealed that she had been addicted to benzodiazepines and chloral hydrate. She reported using in excess of 50 ml of 10 g/% chloral hydrate daily between 1975 and 1988. Sedation treatments were stopped in 1988, so that her medication in 1994 comprised dyazide, fosinopril for hypertension, thyroxine, lithium carbonate for depression, and oestrone hormone replacement therapy. A plain abdominal radiograph showed a normal intestinal gas pattern. Pale white plaques, the histology of which was reported as showing non-specific changes, were noted in the sigmoid and descending colon at colonoscopy. There were no cysts. The faeces were strongly methanogenic, and breath CH_4 concentration was greater than 20 ppm above ambient on two occasions. Fasting breath H_2 was less than 10 ppm on these occasions.

PATIENT 3

This woman (date of birth 14 January 1923) had a past history of uncomplicated duodenal ulcer disease for which she was prescribed a course of ranitidine in 1981. In 1984, she was prescribed valium 5 mg daily and chloral hydrate (10 g/%) 12 ml nocte for an anxiety disorder. A chest radiograph was normal in 1988. In 1991 when investigated by colonoscopy for abdominal bloating, she was documented as having severe left sided PCC. This was not treated. Chloral hydrate was ceased in September 1993. A second colonoscopy in March 1996 showed no cysts and was reported by the colonoscopist as showing "plaques of white tissue resembling deflated air pockets". Her medication at that time comprised atenolol, doxepin, temazepam, clonazepam, donnatal, and ranitidine. She went on to have "triple" therapy to eradicate *Helicobacter pylori*. Six weeks after completion of triple therapy, she was seen by the author. Fasting breath CH_4 was equal to ambient CH_4 , breath H_2 was 4 ppm, and her faeces were non-methanogenic (<20 ppm after four hour anaerobic culture).

PATIENT 4

This woman (date of birth 5 June 1915), with a history of epilepsy since the age of two and mild mental retardation, was one of just three patients with PCC diagnosed by colonoscopy at the Princess Alexandra Hospital between

1987 and 1996. These patients were ascertained by Dr Andrew Pascoe, who searched the hospital's pathology records under the diagnostic heading of PCC. Patient 4 was prescribed chloral hydrate (10 g/%) 10–20 ml nocte for sleep in addition to phenobarbitone and dilantin for epilepsy.

PATIENT 5

This male patient (date of birth 21 February 1911), with a past history of bilateral inguinal hernia repair, benign prostatomegaly, and mycosis fungoides, took oxazepam and chloral hydrate to sleep.

PATIENT 6

Detailed clinical records are not available for this female patient (date of birth 3 January 1939), but pharmacy sheets from the Princess Alexandra Hospital indicated that she also was prescribed chloral hydrate.

STUDY OF EFFECT OF ALKYL HALIDES ON FAECAL H₂ AND CH₄ METABOLISM

In vitro human faecal studies

Stools²³ were collected from four CH₄-producing subjects (three healthy volunteers and patient 2) and three non-methanogenic subjects (two healthy volunteers and patient 3). Buffered 5% (w/v) faecal suspensions were prepared within two hours of collection and cultured anaerobically for 24 hours to mimic the substrate-poor conditions of the left colon.²³ Cultures were grown in gas-tight butyl rubber-stoppered glass bottles. The concentration of bacteria in the anaerobic 5% (w/v) faecal batch cultures falls between the concentration of anaerobic bacteria in the caecum and rectum.³² Chloral hydrate, trichloroethanol, trichloroacetic acid, trichloroethylene, or chloroform was added at different concentrations (1, 10, 10², 10³, 10⁴, 10⁵, and 3×10⁵ μM) to the buffered anaerobic faecal cultures. Control cultures received Hepes buffer only. H₂ was measured electrochemically, and CH₄ was determined by gas chromatography on 1 ml gas samples aspirated from the culture headspace at 4 and 24 hours.²³ A CH₄-producing—that is, methanogenic culture—was defined by a headspace CH₄ concentration greater than 1000 ppm at four hours, and non-methanogenesis by a headspace CH₄ concentration of less than 20 ppm at four hours.

In vivo animal experiment

Four adult female Wistar rats (200–250 g) were given chloral hydrate (200 mg/%) in their drinking water. These rats, which came from the University of Queensland Central Animal Breeding House, do not harbour significant methanogens. Net faecal H₂ production (as inferred from headspace H₂ concentration) was measured in four hour cultures 3, 8, 15 and 22 days after commencement of the chloral hydrate regimen, and was compared with net faecal H₂ production in littermate female controls kept in separate cages. Two rats from each group were killed on day 23 by a lethal inhaled dose of isoflurane and then intestines were removed for macroscopic examination. They

were Swiss-rolled, fixed in formalin, embedded in paraffin, sectioned, and stained for light microscopic examination by a trained histopathologist. The remaining rats were then switched to the control drinking regimen—that is, tap water—but continued to be caged separately.

STATISTICAL ANALYSIS

Instat non-parametric analysis software (Instat version 2.01 for MacIntosh) was used to determine statistical significance of differences between sample means. Significance was assumed if two-tailed p<0.05.

ETHICAL APPROVAL

The project was approved by the Mater Adult Hospital Ethics Committee, which is constituted according to Australian NH&MRC guidelines.

Results

With respect to methanogenic control cultures, mean H₂ concentration at four hours was 180 ppm, and at 24 hours it was 10 ppm or less. Mean CH₄ at four hours was 2100 ppm, and at 24 hours it was 6500 ppm (fig 1A). With respect to non-methanogenic control cultures, mean H₂ concentration at four hours was 900 ppm, and at 24 hours it was 550 ppm. Mean CH₄ at four hours was 6 ppm, and at 24 hours it was 10 ppm (fig 1B). Thus there was on average at four hours, five times more H₂ in non-methanogenic cultures, and at 24 hours, more than 55 times more H₂ in non-methanogenic cultures compared with methanogenic cultures.

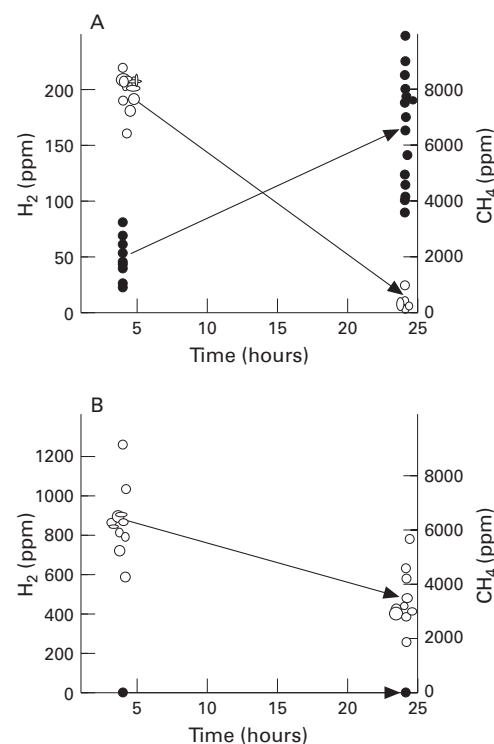


Figure 1: H₂ and CH₄ (ppm) in headspace of control faecal cultures. A, Methanogenic cultures (n = 24, four subjects × six cultures). B, Non-methanogenic cultures (n = 18, three subjects × six cultures). Filled circles = CH₄ ppm, open circles = H₂ ppm. Note different scales for H₂.

TABLE 1 Maximum H_2 in cultures amended with chloral hydrate or trichloroethanol, and trichloroacetic acid or trichloroethylene

Culture	Ch/Tce	TCA/TCE
CH_4		
Mean	240	55
Range	(100–900)	(40–90)
Non- CH_4		
Mean	3.8	2.0
Range	(2–10)	(1.8–3)

Data are means (range) of maximum H_2 measurements ([alkyl halide] = 1–10 mM) normalised to mean control culture H_2 for each experiment: CH_4 = methanogenic cultures (n=16), non- CH_4 = non-methanogenic cultures (n=12). Ch, chloral hydrate; Tce, trichloroethanol; TCA, trichloroacetic acid; TCE, trichloroethylene.

The alkyl halides increased net H_2 production in a concentration dependent fashion with the exception of in those non-methanogenic cultures with very high concentrations of alkyl halides. (With [alkyl halide] $\geq 10^5 \mu\text{M}$ in non-methanogenic cultures, H_2 and CH_4 were depressed below control levels at 4 and 24 hours.) Increased bacterial production of H_2 was apparent from the lowest concentration tested with chloral hydrate or trichloroethanol ($\geq 1 \mu\text{M}$), and for [trichloroacetic acid or trichloroethylene] $\geq 100 \mu\text{M}$ and was maximal at [alkyl halide] = 10^3 – $10^4 \mu\text{M}$. Chloral hydrate or trichloroethanol (1 – $10^4 \mu\text{M}$) increased H_2 equally ($p = 0.45$) over control levels and four times more than trichloroacetic acid or trichloroethylene in methanogenic cultures ($p = 0.02$), and two times more than trichloroacetic acid or trichloroethylene in non-methanogenic culture experiments ($p = 0.03$). There was no difference detected between H_2 concentrations in methanogenic faecal cultures derived from a former PCC subject versus methanogenic faeces from non-PCC subjects or non-methanogenic faecal cultures derived from a former PCC subject versus non-methanogenic faecal cultures from non-PCC subjects ($p > 0.45$). Table 1 shows mean maximum H_2 for methanogenic and non-methanogenic culture experiments. Data are expressed as a ratio of H_2 in alkyl halide-amended cultures to that in unamended control cultures. For methanogenic cultures with chloral hydrate or trichloroethanol (n = 8), mean maximum H_2 production was 240 times control levels and for non-methanogenic cultures (n = 6) 3.8 times control levels. Trichloroacetic acid or trichloroethylene increased net H_2 55 times in methanogenic cultures (n = 8), and 2.0 times control H_2 levels in non-methanogenic cultures (n = 6). The differences in maximum net H_2 between each of the groups were statistically significant ($p \leq 0.03$). In a single experiment with chloroform using faeces from non-PCC donors, H_2 was increased 180 times in a methanogenic culture (n = 1) and 1.9 times control levels in a non-methanogenic culture (n = 1).

Figure 2 shows mean H_2 in alkyl halide-amended cultures relative to H_2 in control cultures at four hours, versus alkyl halide concentrations (1 – $10^5 \mu\text{M}$). K_i values were calculated from data pooled from all methanogenic and non-methanogenic cultures for each alkyl halide and by assuming that the kinetics of

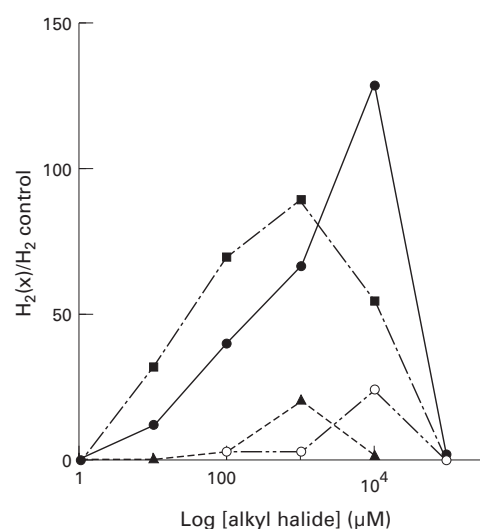


Figure 2: H_2 versus alkyl halide (concentration range $1 \mu\text{M}$ – 100 mM). Points are means of normalised measurements at four hours from both methanogenic and non-methanogenic culture experiments (n = 28). Data are expressed as the ratio of H_2 (ppm) in alkyl halide-amended cultures to mean H_2 (ppm) in unamended control cultures. H_2 values were significantly greater than control net H_2 for chloral hydrate (closed circles), trichloroethanol (closed squares) ($[x] \geq 10 \mu\text{M}$), for trichloroacetic acid (closed triangles) ($100 \mu\text{M} \leq [x] \leq 1 \text{ mM}$) and for trichloroethylene (open circles) ($100 \mu\text{M} \leq [x] \leq 10 \text{ mM}$).

inhibition of bacterial consumption of H_2 was linear over the four hours. The K_i for chloral hydrate, trichloroethanol, chloroform, trichloroethylene, and trichloroacetic acid were respectively 0.2, 0.03, 0.3, 0.4, and 3 mM.

Inhibition of CH_4 formation in methanogenic cultures by alkyl halides was maximal at [alkyl halide] = 10^3 – $10^4 \mu\text{M}$, corresponding to the alkyl halide concentration that resulted in maximum H_2 . At this concentration, chloral hydrate and trichloroethanol both depressed CH_4 levels to 110 (50–640) times less than control levels ($p = 0.005$), and trichloroacetic acid and trichloroethylene depressed CH_4 levels 6 (4.5–10) fold ($p = 0.009$). Methanogen bacteria produce 1 mol of CH_4 from oxidation of 4 mol of H_2 . Thus the expected ratio of excess H_2 to deficit CH_4 caused by inhibition of H_2 oxidation by methanogens is 4, but the experimental mean of excess H_2 /deficit CH_4 was 10.4. There was considerable intra-experimental variation in this ratio, which was not statistically significant for the alkyl halide species or their concentration (0.1–10 mM). The median (range) of excess H_2 /deficit CH_4 was 7 (0.6–31).

The Wistar rats that had chloral hydrate added to their drinking water, drank on average 8 ml fluid/day compared with 11 ml/day by controls. The behaviour of the rats that drank the chloral hydrate ($\leq 0.08 \text{ g/kg}$ per day)-amended water did not appear to be different from controls—that is, they were not drowsy or less active, did not lose weight or condition, and did not develop altered bowel habit over 23 days of exposure to chloral or during the subsequent washout period. Net faecal H_2 production by rats fed chloral hydrate was 10 times that of controls from day 3 (the first day faecal pellets were collected after commencement of

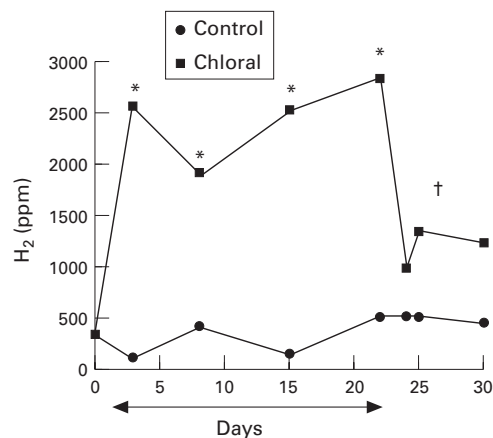


Figure 3: Faecal H₂ production by rats exposed to chloral hydrate. Points are mean H₂ in headspace of four hour faecal cultures derived from rats exposed to chloral hydrate (≤ 0.08 g/kg per day) versus negative controls. * $p \leq 0.04$ for comparisons between chloral and control groups. For the washout period immediately after the end of chloral hydrate treatment, differences between mean H₂ of the washout group and the negative control group, or previous chloral group, were not statistically significant for individual days but were so when means were pooled over three days, † $p = 0.02$ for difference between chloral (day 23) and washout (days 24–30) and * $p = 0.05$ for difference between washout (days 24–30) and control (days 24–30).

chloral hydrate) and throughout the period of chloral administration (fig 3). Macroscopic and microscopic examination of intestines from two rats from each group did not show pneumatosis or any other significant finding. Net faecal H₂ production fell towards control levels during the washout period, although they remained significantly elevated over the period of observation.

Discussion

The principal findings of this study were (a) the striking clinical observation that six consecutively ascertained PCC patients were taking chloral hydrate at the time of their diagnosis, (b) the greater H₂ consumption by methanogenic than non-methanogenic human faecal cultures, (c) the dramatic increase in net H₂ production by in vitro human faeces with added alkyl halides, (d) the parity of maximum net H₂ production by methanogenic and non-methanogenic faeces with added alkyl halide, (e) the reciprocal decrease in CH₄ production by methanogenic faeces with alkyl halide, and (f) the dramatic increase in net H₂ production by faeces, which resulted from chloral hydrate ingestion in the in vivo rat model. The major negative finding of the study was the absence of pneumatosis cystoides in the rat model.

Of the six consecutively ascertained patients with PCC, all were taking chloral hydrate. There is a report in the PCC literature³³ of a patient who was noted to be taking chloral hydrate. This also happens to be the only reported instance of familial PCC. It involved a mother, who was taking chloral hydrate, and her son, who had a psychiatric illness. There is also the hitherto unexplained epidemiological relation between exposure to trichloroethylene

and PCC in Japan.³¹ Trichloroethylene is metabolised in the liver to trichloroethanol, the main active metabolite of chloral hydrate.

The bacteria in control faecal cultures produced H₂, which by 24 hours was consumed in methanogenic cultures (fig 1A), but not in the non-methanogenic faecal cultures from humans (fig 1B). The methanogenic cultures were about 55 times more efficient at consuming H₂ than non-methanogenic cultures. This is consistent with data from most research workers in the field, who have observed methanogenic faecal bacteria to consume H₂ more efficiently than non-methanogenic faecal bacteria,^{22–24 34} and methanogenic humans to excrete less H₂ than non-methanogenic humans.³⁵ The principal H₂-consuming bacteria in the human colon are methanogens in methanogenic individuals^{23 34} and acetogens in non-methanogenic humans.^{22 24} The latter are less efficient at H₂ consumption than methanogens, which explains why non-methanogenic humans produce more net H₂ than methanogenic humans.

Net H₂ was increased many-fold over control levels in the in vitro faecal cultures exposed to various alkyl halides (≥ 1 μ M), although at very high concentrations (≥ 100 mM), the data show that H₂ was depressed in non-methanogenic faecal cultures. Reports of methanogenic (but not acetogenic) consortia, or even pure methanogen cultures, catabolising alkyl halides such as chloroform³⁶ might explain why the methanogenic (but not non-methanogenic) faecal cultures in this study tolerated very high concentrations of the alkyl halides.

These alkyl halides with molecular masses from 119 Da (chloroform) to 165 Da (chloral hydrate) are chemically similar. Chloroform is known to inhibit acetogens³⁷ and methanogens.³⁶ In this study, there was a profound inhibition of methanogenesis in concert with the increase in net H₂. Thus the experimental data suggest that alkyl halides of comparable molecular mass behave like chloroform. Although acetogenesis was not measured, its inhibition by alkyl halides can be inferred because there was a significant increase in net H₂ produced by the non-methanogenic cultures with alkyl halides. The data are also consistent with the occurrence of acetogenesis in methanogenic cultures²⁴ because the ratio of excess H₂/deficit CH₄ was often in excess of 4 (the expected stoichiometric ratio for methanogenesis).

The 10-fold increase in net H₂ production by faeces, which resulted from chloral hydrate ingestion in the rat model (fig 3), occurred in non-methanogenic faeces. This is quantitatively similar to the situation with the in vitro non-methanogenic human faecal cultures. The in vivo effect was apparent within three days of commencement of the chloral hydrate regime and was partially reversed within one day of stopping it.

Can it be inferred from these data that ingestion of chloral causes reversible super H₂ production in humans? The answer must surely be in the affirmative. Patient 2 admitted

to ingesting greater than the recommended prescribed dose of chloral hydrate, which is addictive and subject to tachyphylaxis. By assuming that trichloroethanol has a volume of distribution equivalent to total body weight, it is calculated that the peak concentration of trichloroethanol in the colon, which could result from patient 2 ingesting 0.1 g chloral hydrate/kg per day, is equal to 0.6 mM. This is in excess of the experimentally determined K_1 for chloral hydrate and trichloroethanol. The assumptions that are behind this calculation are supported by the experiment in Wistar rats. They ingested 0.08 g chloral hydrate/kg per day or less, and readily became super H_2 producers with H_2 production quantitatively equal to H_2 production by in vitro non-methanogenic human faecal cultures with alkyl halides. Thus it is readily envisaged that habitual ingestion of chloral hydrate could lead to inhibition of H_2 consumption by colonic acetogen and methanogen bacteria thereby causing super H_2 production and creating the conditions for counterperfusion supersaturation.

Alkyl halides did not enhance H_2 in non-methanogenic as much as in methanogenic faecal bacterial cultures (table 1). However, H_2 concentrations were already 5–55 times greater in the control non-methanogenic cultures (fig 1), so that H_2 concentrations were nearly identical when bacterial consumption of H_2 was maximally inhibited in the two groups. These data are consistent with other studies which have shown that non-methanogenic cultures are inherently less efficient at consuming H_2 than methanogenic cultures,^{22–24 34} but that the total production of H_2 by bacteria in the two culture groups is similar.²³ Thus exposure to these alkyl halides makes it possible for super H_2 production to occur in either methanogenic or non-methanogenic faeces. This conclusion that super H_2 production can occur in either methanogenic or non-methanogenic humans who are exposed to alkyl halides, although superficially at odds with the established view that patients with PCC are non-methanogenic,^{1 9 17} is supported by the in vitro work and by patients 1 and 2, who were methanogenic. (Patient 3 was non-methanogenic.)

In conclusion, exposure to chloral hydrate or related alkyl halides such as trichloroethylene is an important cause of super H_2 production in patients with PCC, and conversely the sharp decline in prescription rates for chloral sedation in Australia and elsewhere might explain the extreme rarity of PCC in the recent past. There may be other treatments in the pharmacopoeia which, by inhibiting bacterial consumption of H_2 , could also cause super H_2 production. Neomycin may be an example. It is a poorly absorbed antibiotic and is one of the few antibiotics that inhibit methanogens.³⁶ It has been described in association with PCC.³⁸ Bile acids spilling over into the colon might also be considered in the aetiology of PCC. Bile (acid) inhibits bacterial consumption of H_2 by methanogens and other H_2 -consuming bacteria in vitro,^{23 32} thus greatly increasing H_2 . However, because bile acid accelerates colonic

transit, which would wash out H_2 -producing bacteria, the conditions for PCC would not normally occur in the diarrhoeal state associated with excess bile acid. In this respect, it is pertinent that colonic transit has been shown to be normal or even slow in patients with PCC¹⁴ despite the clinical symptoms of loose bowel movements and mucus.

It remains for us to consider the cause of secondary pneumatosis cystoides affecting the small intestine in immunocompetent patients. In the series of Elliot,⁷ pyloric obstruction occurred in 60% of cases, but the gas cysts were described in the jejunum.¹ In the detailed case description of Reinhoff and Collins,³ the cysts were distal to the obstruction caused by a Crohn's stricture. In both descriptions, gastrointestinal obstruction was located proximally to the gas cysts. It is hypothesised that the two important aetiological factors in secondary pneumatosis cystoides are the presence of a proximal obstruction^{1–3 7} (or pseudo-obstruction^{8 10}) and H_2 production from bacterial overgrowth.³⁹

In health, about 15 ml of air is ingested with each swallow, and the portion of the swallowed gas that is not belched passes into the small intestine,^{27 40} from where it is absorbed. N_2 entering the lumen would normally dilute gut luminal H_2 which is produced in the setting of bacterial overgrowth. Where gut obstruction occurs proximal to a locus of bacterial overgrowth, N_2 cannot enter via the lumen. If there is also a relative diffusion barrier to blood borne N_2 (see ref²⁰)—a probable circumstance in elderly patients with vascular disease or patients with connective tissue disease^{3 4 8 9}—then H_2 tension in the gut lumen could become equal to N_2 tension in the blood. In this setting, counterperfusion supersaturation of gases will occur in the gut wall, provided that the inherent unsaturation of tissue is small.²⁰ Metabolism of O_2 , which desaturates gas tension in tissue,⁴¹ is the safety factor that could prevent gas bubbles forming more often. We are currently examining the effect of reduced O_2 tension in the chloral hydrate rat model. Normal O_2 tension may explain the absence of PCC both in some persons with high luminal H_2 ²¹ and in the rat model in spite of super H_2 production, whereas low O_2 tension in blood, which would result in small inherent unsaturation in tissue, could explain clinical associations between chronic lung disease^{6 9} or vascular disease^{3 4 8 9} and PCC.

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