Progress report

Breath-analysis tests in gastroenterology

Though the term 'breath-analysis test' conjures up images of policemen and errant motorists, it has a different connotation when used by gastroenterologists. Analysis of certain constituents in breath exhaled after the administration of various substrates has been found to have potentially important applications, and interest in breath tests is increasing for a number of valid reasons.

First of all, in some cases the gases measured are produced in chemical reactions that are difficult to quantitate in any other way, because they take place either inside cells or in the inaccessible regions of the gastrointestinal tract. Secondly, exhaled breath is easy to collect by simple, non-invasive, and aesthetically acceptable means, often using disposable or cheap apparatus readily adapted to local hospital or even field conditions. Thirdly, serial studies can be performed in order to assess the course of a disease or its response to treatment without causing pain or hardship. Fourthly, the analysis of the breath specimens is simple, automated, and potentially highly cost-effective. Fifthly, breath tests do not depend on good pulmonary function for their accuracy to the same degree that tests involving, for example, urine depend on accurate collections and on good renal function. This is because the gases being sought diffuse readily across biological membranes down concentration gradients, pass into plasma and thence into alveolar air. Admittedly, severe metabolic or respiratory acid-base problems would interfere with a breath test, but in the presence of these abnormalities such tests probably would not be performed. Finally, breath tests are safe for use in adults and some of them are safe for use in children.

In this report each of the exhaled gases of gastroenterological interest will be considered and the present methods, applications, and areas of potential usefulness will be reviewed.

\[14^C\text{ Carbon Dioxide (}14\text{CO}_2\)\]

**TOTAL CO\textsubscript{2} COLLECTION METHOD**

The end products of metabolism of all nutrients include water and carbon dioxide. Soon after \[^{14}C\] became available, substrates labelled with this isotope were produced and studies of metabolism were carried out on a variety of nutrients, drugs, and other chemicals, in which labelled precursors were followed to \[^{14}\text{CO}_2\] and measured by alkaline precipitation as \[\text{Ba}^{14}\text{CO}_3\] and counting of the solid phase\footnote{1}.

More accurate assessment of \[^{14}\text{CO}_2\] production became possible with the development of devices that measured total \[\text{CO}_2\] by infrared gas analysis, and \[^{14}\text{CO}_2\] contained therein by an ionization chamber\footnote{8}. Such instruments have been used to follow minute by minute changes in the exhalation of \[\text{CO}_2\] and of \[^{14}\text{CO}_2\], to study the body's \[\text{CO}_2\] and HCO\textsubscript{3}– pools, and to devise mathematical models to explain the curves obtained after substrate injection in terms of kinetics and compartments\footnote{8}.
A total breath collection apparatus was developed for use in clinical situations and formal breath tests of intestinal absorption of nutrients were performed with it. It is a complicated piece of machinery which, though commercially available, has always remained a research tool. Early studies showed that subjects who digested and absorbed fat normally could be distinguished from those with steatorrhoea due to various causes by measuring the $^{14}\text{CO}_2$ exhaled after an oral dose of a medium-chain triglyceride (MCT) trioctanoate-1-$^{14}\text{C}$.

A medium-chain triglyceride was chosen because its constituent fatty acids are absorbed directly into the portal system and are metabolized rapidly. Since it was subsequently shown that medium-chain triglycerides could probably be absorbed without undergoing full hydrolysis, they are not suitable as substrates in tests of fat absorption, and most subsequent work has been done with long-chain triglycerides, structurally resembling the normal constituents of dietary fat. A number of French and German workers, including Grenier et al, Glaubitt, Uthgenannt, and Schneider et al studied patients with a variety of gastrointestinal and endocrine disorders, and rats with experimental pancreatitis, using the technique of total $\text{CO}_2$ and $^{14}\text{CO}_2$ collections and measurement after the administration of $^{14}\text{C}$ labelled fatty acids and triglycerides, but the technique has not been widely adopted in clinical gastroenterology. Total collection methods are used in studies in experimental animals.

**Interval sampling of $\text{CO}_2$**

Breath tests were greatly simplified by Abt and Von Schuching who showed that since the sedentary subject produced a roughly constant amount of $\text{CO}_2$ per unit time, interval sampling of exhaled breath was sufficient for measuring $^{14}\text{CO}_2$ output. They used a simple arrangement, with the subject exhaling directly through a calcium chloride drying chamber into a vial containing a measured volume of hydroxide of hyamine and a phenolphthalein indicator. At the point of the colour change, knowing the volume and the molarity of the hyamine, one could calculate the amount of $\text{CO}_2$ 'trapped', add scintillant directly to the vial, and determine $^{14}\text{CO}_2$/mmol $\text{CO}_2$. By performing such collections at intervals following the administration of a labelled substrate and by assuming an average rate of $\text{CO}_2$ output per unit time, either empirically or based on weight or surface area, one can estimate the total amount of the administered $^{14}\text{C}$ exhaled during a period of observation. This assumption is convenient, but it is subject to the problems and inaccuracies that render basal metabolic rate testing an unsatisfactory procedure.

The change from total collections to interval sampling has meant that breath tests in which $^{14}\text{CO}_2$ is measured can be performed without elaborate apparatus, even under field conditions. The breath-sampling apparatus can be made in a few minutes out of disposable components. It has been estimated by one manufacturer that there are 13 000 liquid scintillation counters in the world—excluding those used for teaching purposes—so it is reasonable to assume that anyone wishing to perform such breath tests should be able to arrange access to a counter and to proceed with little capital outlay.

$^{14}\text{CO}_2$ and the malabsorption of fat

Kaihara and Wagner applied this method formally to the study of fat absorption. They administered 5 $\mu\text{Ci}$ of glyceryl tripalmitate-1-$^{14}\text{C}$ in a test
meal of 1 g fat/kg body weight, sampled breath hourly, and measured peak exhalation expressed as a percentage of administered specific activity per mmol CO₂. The peak occurred between the third and sixth hour following administration, implying that the test would be lengthy, especially when performed on outpatients. Despite a narrow zone of overlap, there was good separation of normal subjects and patients without steatorrhoea from patients with steatorrhoea caused by diseases or by neomycin. Carbohydrate must be restricted during the test for it to be valid and patients with metabolic abnormalities, such as fever, thyroid disease, and diabetes, must be excluded. It has been shown that misleading results are obtained in obese patients; they exhale less specific activity per mmol CO₂ than might be predicted, presumably because the labelled fat is diluted into a large pool of unlabelled body fat.

Few reports dealing with the glyceryl-tripalmitate-1-14C test have appeared in the literature since Kahiara and Wagner’s paper. It was reported on favourably in two publications from Belfast15,16 which included scant clinical data for the patients studied. A more recent assessment supports its usefulness as a screening procedure, particularly in cases of suspected pancreatic insufficiency, but does not endorse using it as a replacement for formal faecal fat studies17.

The collection and measurement of stool fat is expensive, time consuming, and often inaccurate. A screening test for malabsorption is highly desirable, but is unlikely to replace entirely the more formal chemical measurement. If the screening test were reliable enough merely to identify positively as normal or abnormal any relevant group of patients and thus decrease by only 20% the number of stool collections and estimations, it would represent a considerable saving of resources. Careful further assessment of this procedure is therefore to be encouraged.

Many 14C-labelled fatty acids and triglycerides are commercially available and have been used to define precisely the absorptive defects in a variety of malabsorptive disorders18.

14CO₂ AND MALABSORPTION OF LACTOSE
Among the earliest studies of 14CO₂ production following the administration of a labelled substrate was that of Cozzetto19 who showed that the administration to a child with lactose intolerance of 5 μCi of 14C-lactose and a large carrier dose of unlabelled lactose resulted in exhalation of little 14CO₂. Several years later two groups20,21 using interval sampling techniques showed that lactose-intolerant subjects could be differentiated from lactose-tolerant ones by measuring 14CO₂ after oral administration of 5 μCi of lactose-1-14C in a 50 g carrier dose of unlabelled lactose. The advantage of this technique over lactose tolerance tests, mucosal disaccharidase assay, lactose-barium small bowel x-ray series, or breath H₂ measurement22,23 following lactose ingestion is that it is the most adaptable to field work and epidemiological studies. Recovery of patients with secondary hypolactasia, as can occur in the coeliac syndrome or malnutrition, may be followed in a simple way with this test.

14CO₂ AND MANNUITOL
Mannitol is a poorly absorbed alcohol, closely related in chemical structure to mannose. It has long been known to be metabolized by bacteria, and has
been used clinically as an osmotically-active laxative. Salmon et al\textsuperscript{24} reported that in patients with bacterial overgrowth of the small bowel, oral administration of a trace of mannitol-1-\textsuperscript{14}C in 2.5 g of unlabelled mannitol was followed by the prompt appearance of $^{14}$CO\textsubscript{2}. Subjects whose intestines were not colonized did not metabolize and absorb the mannitol or exhale $^{14}$CO\textsubscript{2}. They found the test was a more sensitive indicator of the blind loop syndrome than was the measurement of urinary indican following a loading dose of 1-tryptophan. The test has not been reported on by other authors.

$^{14}$CO\textsubscript{2} AND BILE SALTS
Many patients with ileal disease or who have undergone ileal resection develop hyperoxaluria\textsuperscript{25}. Such patients also have interruption of the enterohepatic circulation of bile salts, entry of increased amounts of these salts into the colon where they are deconjugated and in part lost in the stools, and a compensatory increase in hepatic synthesis of bile acids conjugated to glycine and taurine. Since glycine is much more abundant than taurine, the ratio of glycine conjugates to taurine conjugates rises in the bile of these patients\textsuperscript{21}.

An hypothesis to link the hyperoxaluria to increased bile salt deconjugation suggested that the increased load of glycine conjugates in resected patients spilling into the colon and being deconjugated resulted in an increased load of glycine being metabolized through the glyoxalate to oxalate pathway, thus leading to hyperoxaluria\textsuperscript{28}. To test this idea, Hofmann's group synthesized glycine-1-$^{14}$C-cholate, administered it to patients with ileal resection, and monitored urinary and breath radioactivity\textsuperscript{29}. The urinary results were equivocal, but subjects with ileal resections clearly exhaled more $^{14}$CO\textsubscript{2} than did normal controls. Hofmann et al suggested that breath testing might be a simple means of assessing the integrity of the enterohepatic circulation, and thus be of use in diagnosing ileal disease or blind-loop syndromes.

The underlying assumptions in this breath test are that bile salts can be deconjugated into bile acids and amino acids by enzymes found only in microorganisms; that this cleavage results in the absorption of the amino acid which is then rapidly metabolized and the $^{14}$CO\textsubscript{2} exhaled, or, alternatively, that the amino acid is metabolized intraluminally and the $^{14}$CO\textsubscript{2} absorbed and exhaled, and that normal subjects with intact enterohepatic circulations do not metabolize the conjugated labelled amino acid. Much evidence has been produced to support each of these assumptions. No mammalian cell yet studied is capable of deconjugating bile salts, while many of the bacterial strains that are present in the colon are capable of deconjugating them. These bacteria have been extensively studied and have recently been reviewed by Lewis and Gorbach\textsuperscript{37}. The metabolism of glycine, which is absorbed actively in the small intestine and passively in the colon, has also been well investigated\textsuperscript{28}, as has the bacterial metabolism and degradation of this amino acid. The bile acid breath test was developed by Hofmann's group\textsuperscript{39} and by Sherr et al\textsuperscript{40} with similar results: namely, that breath testing of the integrity of the enterohepatic circulation was simple and convenient. Abnormal results were obtained in subjects with an interrupted enterohepatic circulation whether due to ileal resection or to proximal intestinal overgrowth with bacteria.

The test is performed by administering 5 $\mu$Ci of the labelled bile salt either with, or just before breakfast, and sampling breath hourly thereafter. Normal
subjects have very little rise in specific activity per mmol CO₂ for several hours, but do lose appreciable amounts of the label in the breath with each circulation of the bile acid pool, and half the label if sampling is continued for up to a week. In contrast, patients with an interrupted enterohepatic circulation lose much more of the label more rapidly. Fromm and Hofmann distinguished a normal group from an abnormal group on the basis of breath collected at two and four hours after ingestion of the labelled bile acid, while Sherr et al calculated the total exhaled in six hours. Our experience suggests that in all cases in which excessive deconjugation can be demonstrated, it becomes quite obvious by the end of the third hour following administration of the label. A recent report confirms this, but suggests continuing the test for a total of four hours.

Several groups have confirmed the clinical usefulness of this test and it is gaining widespread acceptance. In addition to all the usual causes of proximal intestinal bacterial overgrowth—which have recently been reviewed—the test also gives a positive result in certain cases of cholangitis and liver disease. In theory it will give a positive result if the labelled bile acid is deconjugated by bacteria before administration, or in the mouth, oesophagus, or stomach. The test is positive in certain cases of Crohn’s disease of the terminal ileum, though it is difficult to know whether this is due to a deficiency of normally functioning ileum, or to stagnant loop syndrome proximal to a partially obstructed intestinal lumen. Breath testing alone does not distinguish between ileal resection and proximal intestinal overgrowth, and in the situation where such a question needs an answer, the counting of the faeces passed during the 24 hours following administration of the labelled bile acid may give additional useful information. Fromm et al have found that about two-thirds of subjects with ileal resection excreted excessive faecal ¹⁴C, while excessive faecal radioactivity was absent in all the patients who had proximal bacterial overgrowth.

The sensitivity of the test was studied by comparing it to anaerobic culture of the small bowel and to the Schilling test of vitamin B₁₂ excretion. It has been found that the breath test was abnormal in a number of patients with ileal resection, diarrhoea, and normal Schilling test results, while few patients with abnormal B₁₂ absorption had normal breath tests.

Discrepancies between the breath test and anaerobic culture of intestinal contents have occurred, but corroborative evidence of the existence of a blind loop syndrome in the presence of a normal breath test could not be obtained. In one patient with unequivocal clinical and x-ray evidence of a blind loop syndrome, a positive breath test, and a therapeutic response to tetracycline, the anaerobic culture was sterile. Though the breath test will never fully replace carefully performed anaerobic culture in the diagnosis of blind loop syndromes, it is so easy, cheap, and reproducible that it deserves widespread application as an important screening test for this condition.

It is usually accepted that bile salts escaping the enterohepatic circulation and spilling into the colon can cause the diarrhoea of cholerhoeic enteropathy. Hofmann’s group suggested that non-bile-salt-related diarrhoea might cause the passage of excessive amounts of bile salts into the colon, with subsequent deconjugation and false positive breath tests—the clinical counterparts of what Meihoff and Kern found in volunteers in whom diarrhoea was induced by oral mannitol and in whom the half-life of bile salts was shortened. Such a situation is to be suspected in subjects with a positive
breath test who fail to respond to the two available treatments: cholestyramine for resection and broad-spectrum antibiotics for blind loop syndrome. False positive breath tests of this sort must be rare. The Hofmann group's most recent study\(^5\) suggests that though non-bile-salt-related diarrhoeal illnesses may be associated with increased faecal loss of bile salts, they are not usually associated with increased \(^{14}\text{CO}_2\) exhalation.

It should be noted in passing that the original hypothesis linking hyperoxaluria to excessive glycine degradation has now been discarded, and the present theory is that some patients with ileal resection absorb dietary oxalates excessively\(^39,40\).

Similar to other tests of interval \(^{14}\text{CO}_2\) sampling, the bile salt breath test has great potential for epidemiological investigation of patients in whom the integrity of the enterohepatic circulation needs to be ascertained. One such group are women who have received radiotherapy to the pelvic organs. By means of the bile salt breath test we have shown that a very much higher incidence of intestinal side effects occurs as a consequence of such irradiation than had been supposed from retrospective studies of patients suffering from clinically obvious radiation enteritis\(^41\).

\(^{14}\text{CO}_2\) AND PROTEIN CATABOLISM

Interval Measurement of \(^{14}\text{CO}_2\) at intervals has been used in studies of protein catabolism\(^42\). Following the administration of \(\text{Na}_2\)^\(\text{^{14}CO}_2\), the body's \(\text{CO}_2\) pool is quickly labelled. Since this pool equilibrates with the carboxyl group of glutamate and aspartate, proteins also become labelled. Upon their destruction, the amino acids are released and thus \(^{14}\text{CO}_2\) is exhaled: in this manner protein catabolism is reflected by \(^{14}\text{CO}_2\) exhalation. Such studies are of interest in the area of protein metabolism in relation to nutritional status. Similar methodology may be used in investigations of gastrointestinal protein loss.

\(^{14}\text{CO}_2\) AND CHOLESTEROL

In an intriguing series of experiments recently reported by Ginter\(^43\), measurements of breath \(^{14}\text{CO}_2\) were used to study the metabolism of cholesterol in the guinea pig. Cholesterol-26\(^\text{-^{14}C}\) was administered to animals previously rendered subclinically scorbustic by treatment with known inadequate amounts of vitamin C. When compared to normals, the deficient animals exhaled less specific activity per mmol \(\text{CO}_2\). Since the cleavage of \(\text{C}_{\text{q5-q7}}\) is an essential step in the formation of cholic acid, this study suggests that in the guinea pig vitamin C plays a role in bile acid synthesis. The relationship between vitamin C and cholesterol metabolism is controversial\(^44\), and further studies in the guinea pig and in other species are awaited.

\(^{14}\text{CO}_2\) MISCELLANEOUS TESTS

Other tests in which \(^{14}\text{CO}_2\) is measured following the administration of specifically labelled substrates have from time to time been described. Fish \textit{et al}\(^45\) labelled histidine in the two-ring position and found that there was less \(^{14}\text{CO}_2\) produced in folate-deficient subjects than in normal controls or in patients with pernicious anaemia. This test, simpler to perform than the measurement of Figlu, but subject to the same tendency to inaccuracy, has been rendered obsolete by the development of specific assay methods for folate.
Segal et al. found that most galactosaemic infants could not metabolize a trace of galactose-1-14C while normal infants could. It might similarly be predicted that subjects with maple sugar urine disease who cannot decarboxylate oxidatively the branched chain amino acids would not produce 14CO2 from valine, leucine, or isoleucine labelled in the carboxyl carbon. Such a finding has been demonstrated in white blood cells from these patients.

Syndromes associated with thiamine deficiency which are common in areas of poor nutrition and among alcoholics are difficult to diagnose. Of the availability tests, red cell transketolase seems to be the most reliable and the most widely used, but is quite tedious. Transketolase, for which thiamine is a cofactor, is concerned in the hexose monophosphate shunt with the reaction between ribose and ribulose phosphates producing sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate and eventually leading to the production of fructose-6-phosphate which is quickly metabolized. It might be predicted that thiamine-deficient patients would produce less 14CO2 from labelled ribose than would normal people. Brin performed 14CO2 exhalation studies in rats on diets deficient in thiamine, who had been fed 14C-ribose. Fourteen or more days after starting on the diet the rats exhaled less specific activity/mmol CO2 than did control rats. Tests in vitro of another thiamine-dependent enzyme system, ie, pyruvate dehydrogenase, do measure 14CO2 generated from labelled pyruvate, but tests in vivo have not been reported in human subjects and Brin's study of this enzyme in animals is less conclusive.

14CO2 SAFETY

Though sampling of exhaled breath at specific intervals after the administration of 14C labelled substrates has many features that make it desirable for use by gastroenterologists, epidemiologists, and nutritionists, it does involve the ingestion of 5 μCi of radioactive isotope with a long half-life which emits low-energy beta particles (0.05 Mev). The ionizing radiation constitutes a potential hazard to man; a further hazard is the isotope's decay to nitrogen. It is not likely that a smaller dose could be used: the efficiency of the described system of hyamine-trapped CO2 with a toluene-based scintillation cocktail is very high and could not be markedly improved upon. In the bile acid breath test abnormal counts are sometimes only about thrice the background count. Decreasing the administered dose would bring abnormal results too close to the background level for comfort.

On the other hand there are data to suggest that the administration of 5 μCi is not excessively dangerous. Whole-body radiation for widely distributed compounds, such as those used in the tests described above, is quite small. Tolbert and Cozzetto extrapolated the data of Berlin and Tolbert to the administration of 10 μCi of glycine-2-14C to a 70-kg man and found that the 400-day integrated total body radiation dose was about 15 mrads compared with a cosmic and natural radiation dose of 1200 mrads. The radiation exposure on the day of the test is less than 0.4 mrad compared with radiation of 5 mrad gonadal exposure from routine chest film.

Skipper et al. showed in the rat that the injection of NaH14CO3 in a dose equal to about 10 μCi in a 70-kg man gave negligible whole body radiation at the end of 24 hours. In bone, where there is a large bicarbonate pool which exchanges only slowly with other body pools, such a dose would give
a radiation level of 0.03 mrem/day at the end of one or two weeks.

The substances described in the tests of fat, carbohydrate, or bile salt metabolism are all likely to be eliminated from the body quite rapidly. Amino acids cause most concern. It is therefore reassuring to note that Hepner and Hofmann found that subjects with an intact enterohepatic circulation (who would be expected to retain the glycine-1\(^{14}\)C-cholate the longest) eliminated about 60% of the administered dose within one week\(^{64}\).

\(^{13}\)C-enriched substrates are not radioactive and differ from \(^{12}\)C substances only by differences of mass. Such enriched substrates could be used with safety in children and the exhaled CO\(_2\) analysed by mass spectrometry. In a somewhat analogous manner deuterium-enriched bile acids have recently been used in neonates to study bile acid kinetics\(^{58}\). As mass spectrometry and other methods relevant to the use of \(^{13}\)C develop, one may reasonably hope that the use of \(^{14}\)C-containing substances might become unnecessary.

**Carbon Monoxide (CO)**

It has long been known that there is carbon monoxide in small quantities in the blood of normal subjects. Recently it has been shown that in addition to CO acquired from our polluted atmosphere, CO is produced endogenously at the rate of about 6.1 \(\mu l/kg/hr\)\(^{58}\). In newborns, production of CO averages about 16.3 \(\mu l/kg/hr\), while adult females produce CO at a variable rate: during the progestational phase of the menstrual cycle more CO is produced than during the oestrogenic phase.

Several lines of evidence have shown that all CO produced in the body originates from the carbon atom of an alpha-methylene bridge of the protoporphyrin ring of haem molecules. It can be calculated that for every mole of haem catabolized, 1 mole of CO is produced. Study of the production of CO in man has shown that an appreciable percentage of the total must originate from sources other than dying red blood cells. In a manner analogous to that of bile pigments, an 'early labelled' peak of \(^{14}\)CO appears after injection of \(^{14}\)C-glycine. The origin of this early labelled CO is undoubtedly heterogenous and includes the products of ineffective erythropoiesis and the degradation of haem-containing compounds in muscle, liver, and other organs.

The measurement of CO is easy. It can be done in blood by liberating bound CO and measuring the gas in an infrared CO meter. It can be done in breath by gas chromatography, by catalytic combustion, or by infrared CO analysis. Though blood carboxyhaemoglobin concentration is easy to measure, this alone is not a valid method for measuring production of CO, since CO uptake from the environment during the course of the study or even hours beforehand will affect the calculated production rate.

Coburn\(^{57}\) devised a means of measuring the endogenous CO production rate by placing his subjects in a closed rebreathing system, in which the CO\(_2\) produced was absorbed, the oxygen consumed was replaced, and substances such as CO which were being generated endogenously during the course of the study would increase in concentration throughout the system. This is the method used most often to calculate CO production rates in human subjects in a variety of circumstances.

The production of CO parallels bile pigment production: they are measures of haem catabolism. The technique for the study of CO is much simpler than
those of pigment study, and it may become a clinically useful tool in the study of jaundice and of haemolysis.\textsuperscript{68}

\textbf{\textsuperscript{85}Krypton (\textsuperscript{85}Kr)}

Molnar and coworkers have injected \textsuperscript{85}Kr into the spleens of dogs and human subjects and measured its appearance in exhaled air as a measure of portal blood flow. They could readily distinguish between normal dogs and those with portocaval anastomoses.\textsuperscript{69} In human subjects they have found the technique simple and reliable in the investigation of portal blood flow in cirrhosis. Patients with portal vein thrombosis have marked delayed exhalation of the injected \textsuperscript{85}Kr.\textsuperscript{60}

Taking advantage of the great solubility of \textsuperscript{85}Kr in body fat Hytten et al.\textsuperscript{61} have devised a re-breathing apparatus in which by subtracting \textsuperscript{85}Kr dissolved in gas, water, and protein they could calculate the amount administered dissolved in fat. The method seems to be an elegant reproducible way of measuring total body fat.

\textbf{Ammonia (NH\textsubscript{3}), Mercaptans, Dimethyl Sulphide, and Volatile Fatty Acids}

The pK of the equilibrium reaction between NH\textsubscript{4}\textsuperscript{+} and NH\textsubscript{3} is 8.9; at physiological pH almost all circulating ammonia is in the non-diffusible NH\textsubscript{4}\textsuperscript{+} form. In experimental animals, alveolar NH\textsubscript{3} has been measured after the induction of systemic alkalosis.\textsuperscript{62,63} The technique has not been applied to the study of human subjects with hepatic insufficiency but might be an alternative to the Conway microdiffusion method of measuring blood NH\textsubscript{3}.

The mercaptans methanethiol and ethanethiol, believed partly responsible for foetor hepaticus, are present in breath in low concentrations and have been studied both in patients and in normal persons after methionine loading. Dimethyl sulphide (DMS) was also identified in the exhaled breath of these subjects and the intensity of the characteristic odour of foetor varied directly with the concentrations of DMS rather than with that of the mercaptans.\textsuperscript{64}

From the same laboratory, measurements of the volatile fatty acids acetic and propionic acid have been made in breath. As might be expected, the concentration of the volatile fatty acids increases in patients with hepatic insufficiency.\textsuperscript{65}

\textbf{The Intraluminal Gases Hydrogen (H\textsubscript{2}) and Methane (CH\textsubscript{4})}

The presence of H\textsubscript{2} and CH\textsubscript{4} in intestinal gas has been suspected since Magendie\textsuperscript{66} reported a study of guillotined convicts showing these gases to be present in their intestines. The literature since then has contained reports of explosions during colonic surgery and of combustible eructations in patients with gastrocolic fistula,\textsuperscript{67} reinforcing the notion that the gut is a site in which combustible gases may be found. No mammalian cell is capable of producing H\textsubscript{2} or CH\textsubscript{4}; the source of these gases has long been known to be fermentation of appropriate substrates by bacteria under anaerobic conditions.

\textbf{H\textsubscript{2}}

The bean industry has long laboured under the onus that their products
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were ‘wind producing’ and has sponsored research into the origin and nature of bean-related flatulence. Nielsen found that after feeding baked beans to volunteers H₂ appeared in exhaled breath and that the rise in breath H₂ concentration was coincident with his subjects’ abdominal discomfort. He measured H₂ concentration with gas chromatography, a technique which allows the measurement of concentrations of the order of 10 ppm.

Calloway repeated Nielsen’s experiments, showing that breath H₂ measurements were reproducible, substrate related, and correlated to some extent with symptoms. It was further demonstrated in studies in vitro that faecal or ileal-dejecta flora incubated with various substrates produced striking amounts of CO₂ and H₂. When stacchyose, a sugar abundantly present in baked beans, was incubated with ileal or colonic flora as much CO₂ or H₂ were evolved as when glucose, galactose, or other common sugars were incubated. This was of particular interest since stacchyose is an oligosaccharide hydrolysed by an enzyme not present in the intestine and is thus non-absorbable. Enteric bacteria possess this enzyme and are thus able to split stacchyose into fermentable monosaccharides. Another oligosaccharide (raffinose) is also present in beans. The specific hydrolytic enzyme for raffinose is not found in human tissues but the substance is fermentable by the flora and consequently produces intestinal gas. In general terms it is reasonable to say that the wind-producing potential of a food is related to its content of non-absorbable fermentable substrates, most probably oligosaccharidic and fibrous in nature.

Formal studies of the site and rate of H₂ production in the intestine were undertaken by Levitt who perfused various regions of the gut through a multilumen tube. Instead of a liquid perfusion with a non-absorbable water soluble marker, he used a gas (N₂) perfusion with a non-absorbable gas marker. Basally no H₂ was produced anywhere in the intestine. After instillation of a fermentable substrate in normals, H₂ was produced almost entirely in the colon. Subjects with small intestinal bacterial overgrowth did produce H₂ in their contaminated small bowels, but even in these subjects 40 times as much H₂ was produced in the colon. The quantities of colonic H₂ produced were striking: after the instillation of 2 g of lactose, up to 1.5 ml/min were generated. Levitt measured H₂ exhalation and found it was linearly related to intestinal production, averaging 21% of the total.

Both Levitt’s and Calloway’s groups have reported on the use of breath H₂ in the investigation of lactose intolerance with excellent correlation between lactose tolerance tests and breath H₂ measurements. These tests are intellectually satisfying since the malabsorbed sugar being measured is responsible for the symptoms for which the test is often performed. Levitt has shown that as little as 5 g of lactose was followed by a rise in breath H₂ in severely hypolactasic subjects, while Chenoweth has established that a rise in breath H₂ greater than 20 ppm after ingestion of 0.5 g lactose/kg was as accurate as a lactose tolerance test in diagnosing lactose malabsorption. She also found that the amount of lactose absorbed was dose dependent and that there was no detectable H₂ in breath in some lactase-deficient subjects when the test dose was halved, though, as Levitt found, some subjects were exquisitely intolerant to the sugar. On the other hand, many subjects tolerant to 0.5 g/kg exhaled abnormal concentrations of H₂ when retested with 1.0 or 1.5 g/kg of the sugar. The use of serial H₂ measurements in subjects found to be lactase deficient and given lactose-containing diets
for long periods of time may help to resolve the question of whether lactase is an adaptable enzyme. The time of appearance of H₂ in the breath after lactose administration is dose related: the more lactose ingested, the earlier it appears in breath. Since appearance time is a measure of transit from mouth to colon, it is tempting to speculate on the relationship between osmotically active solute loads and transit rates, though there are insufficient data to determine which of these factors is the cause and which the effect.

Bond and Levitt have found that all patients with postgastrectomy diarrhoea who were studied exhaled H₂ after the ingestion of 100 g of glucose, even though they had 'normal' blood glucose curves. Normal subjects and postgastrectomy patients without diarrhoea had no H₂ in the breath after 100 g doses. Since the H₂ producers all absorbed 10 g of glucose, whilst subjects with small bowel bacterial overgrowth exhaled minute but measurable H₂ after this small load of substrate, they reasoned that the H₂ exhaled after the large glucose load in postgastrectomy subjects was colonic in origin.

By administering a measured dose of lactulose, a synthetic non-absorbed disaccharide, and determining the resulting H₂ in breath in each subject and comparing this with the H₂ produced after the glucose load, the amount of glucose malabsorbed was estimated. Subjects with moderate diarrhoea malabsorbed from 13·7 to 17·2 g of a 100 g load, whilst two subjects with severe diarrhoea malabsorbed 15 and 21 g of a 50 g load. By their calculations these figures for malabsorbed glucose are accurate only within 50%, so at best, lactulose calibration is only semi-quantitative.

Chenoweth has used a similar method to quantitate lactose malabsorption in some of her alactasic subjects. Wide application of this method would possibly be of use in estimating the nutritional value of supplementary milk feeding of children likely to be lactase deficient.

Calloway and her colleagues have fairly systematically investigated the gas-forming potential of a variety of fruits, legumes, and cereals. It is fair to say that popular folklore of the wind potential of foods is surprisingly accurate. Apple, grape, and prune juices are all gas producers; orange and apricot juices are not. Pineapples are remarkably free of wind production, and in my experience flatulence-prone individuals frequently select this as their ‘safe’ fruit. Whole wheat and All-Bran cereals produce more total H₂ (breath and flatus) than do refined wheat or bland formula diets. Soya beans and lima beans produce on a weight basis equal amounts of wind and H₂ whilst peanuts do not. Since the fractions of these foods that are fermented are not absorbed by man, the fraction of the total energy represented by fermentable substrate should be subtracted from the total calories contained in the food. The conditions under which foods are grown affects the amount of these substrates, making such calculations exceedingly difficult, but perhaps by widespread application of human assay techniques, such as those used by Nielsen and Calloway in their studies of beans, meaningful average values could be obtained.

The effect of neomycin or succinylsulphathiazole is to increase the gas produced in volunteers following a bean meal, while iodochlorhydroxyquin decreases gas formation in the same situation. Other antibiotics have not been systematically tested. It is perhaps worrying that the two antibiotics most often used in bowel preparation are the ones that allow the production of H₂.

Potential applications of breath H₂ measurements are many and varied.
Hurst, with characteristic prescience, wrote about intestinal carbohydrate dyspepsia 40 years ago and this entity can now be sought with renewed interest. Holdsworth and Dawson found that decreased absorption of glucose in idiopathic steatorrhoea was a highly sensitive test of intestinal function. The application of breath H₂ measurements following glucose administration may prove to be an elegant, simple way of assessing severity or gauging recovery. Reports have appeared relating certain 'irritant' cathartics such as oxyphenisatin or phenolphthalein to glucose malabsorption. Breath H₂ measurements may find a use in the assessment of the mode of action of this class of drugs. Another group of pharmacological substances, the biguanides, have been shown to inhibit intestinal glucose absorption, and though in a preliminary study H₂ did not appear in breath, this important group of oral hypoglycaemic agents might profitably be looked at again in this regard.

Studies of dietary fibre might profitably include measurements of gases produced from bacterial degradation. Bacteria possess the enzymes necessary to hydrolyse two of the principal constituents of fibre, viz, cellulose and hemicellulose, and there is evidence to suggest that most of cellulose is in fact degraded to osmotically active components and, presumably, into gases.

The estimation of exhaled H₂ performed in a systematic manner after ingestion of various substrates may be of great value in the investigation of patients with flatulence who may have been deemed neurotic. Patients with liver disease, who seem very prone to excessive flatulence for poorly understood reasons, may also be profitably investigated.

Though lactose intolerance is now well recognized, carbohydrate malabsorption due to other enzyme deficiencies is considered rare. The clinical entities isomaltase and trehalase deficiency have been described. Recently sucrose malabsorption has been found to be prevalent among Eskimos. Evidence for deficiencies of pancreatic amylase has been sought for by means of the starch tolerance test, but though the results in experienced hands were satisfactory, the test is difficult to perform and not widely used. In each of these examples, breath H₂ measurements following the administration of relevant substrates might help to identify deficient subjects in a simple, painless way.

The role of fermentation in the pathogenesis of neonatal colic has not been well studied, and might be highly relevant. Bottle-fed infants, who suffer more from colic than do breast-fed infants, have different faecal floras and a preponderance of Gram-negative organisms, among which are included the bacteria capable of fermenting sugars. H₂ measurements may be easily performed in infants.

Modern gas chromatographic equipment with molecular sieve columns and thermal conductivity detectors can detect as little as 10-20 ppm H₂ concentration. Machines of this order of sensitivity cost only a few hundred pounds. The exhaled air can be collected in a number of ways. Calloway's group use timed collections in multilaminate plastic bags, the subject blowing through a CO₂ absorber into the bag for several minutes at a time. Levitt et al. use a closed re-breathing system, adapted from the one designed by Coburn and discussed above in the section on CO measurement. Any system which allows collection of the air exhaled in a carefully timed period and which allows calculation of the volume in the system is adequate for most studies of the sort described.
The world's population may be divided into CH₄ 'producers' and 'non-CH₄ producers', with some familial tendency towards CH₄ production⁸⁹, but with no evidence that spouses share the propensity. 'Producers' exhale a concentration of more than 23 ppm while 'non-producers' exhale less than 3 or 4 ppm. CH₄ production never begins before the age of 2. The pattern of CH₄ exhalation is fairly constant in CH₄ producers over the course of a 24-hour day and thus does not seem to be dependent on an exogenous substrate. Indeed the substrates for the CH₄-producing bacteria are unknown and all the bacteria responsible have not been identified. It is known that CH₄ is generated only under strictly anaerobic conditions in the colon. The suggestion has been made that CH₄ results from the reduction of CO₂ with H₂—presumably arising from the fermentative action of other bacteria. The study of intermicrobe interactions is an area of intensive investigation at present and has been recently succinctly reviewed by Bryant⁹¹. Interaction of different strains of microorganisms is believed to be the way CH₄ is produced in sewage disposal systems⁹², which is what the colon, in part, represents.

Production of CH₄ has thus far been found to be relevant only to the flotational qualities of stool: producers have floating stools while non-producers have 'suckers'⁹³. Otherwise this gas does not seem to be related to human disease. It is measurable on a gas chromatograph if a H₂ or He ionization detector is used.

The appreciation that gases produced by bacteria may be present in large volumes in the gut has helped to reshape current thinking on the origins, sources, composition, and disposition of intestinal gas, a subject recently reviewed by Calloway⁸⁴ and by Levitt and Bond⁸⁵.

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