

Breath Tests in Medicine

Since antiquity, it has been known that the breath contains clues to many diseases. Researchers are developing a new generation of breath tests as an alternative to invasive diagnostic procedures

by Michael Phillips

For many people, the idea of breath testing conjures up a vision of a roadside interrogation by a grim-faced police officer. A motorist suspected of being intoxicated must blow into a hand-held instrument that, after a few seconds, indicates the amount of alcohol in the blood. Despite this unflattering association with inebriation and arrest, breath testing is blossoming into an exciting area of medical technology. Physicians have begun using these tests to diagnose an increasingly wide variety of diseases without the hazards or discomforts of invasive procedures. Furthermore, breath tests are providing important new insights into the understanding of basic biochemical functions of the body.

The recent upsurge in interest stems from advances in analytic technology, which have made it possible to identify a rapidly growing number of compounds in the breath. Clinical studies with these improved assays have shown that the presence of abnormal chemicals in the breath can aid in the early diagnosis of many diseases.

The breath contains valuable information because only a slender barrier separates the air in the alveoli of the lung from the blood in the capillaries. This barrier is called the pulmonary alveolar membrane. Like water flowing down a hill, a volatile organic compound, such

as alcohol or methyl mercaptan (the fragrant compound in garlic), will diffuse across the alveolar membrane from the compartment with the higher vapor pressure to the lower, from the air into the blood, or vice versa.

The detection of volatile organic compounds in the breath has a long history. Since the time of Hippocrates, physicians have known that the aroma of human breath can provide clues to diagnosis. The astute clinician is alert for the sweet, fruity odor of acetone in patients with uncontrolled diabetes, the musty, fishy reek of advanced liver disease, the urinelike smell that accompanies failing kidneys and the putrid stench of a lung abscess.

But without objective chemical analysis, breath testing as a medical tool could not have progressed beyond educated sniffing. This problem attracted the attention of scientists more than 200 years ago. Antoine Laurent Lavoisier, celebrated as the father of the "chemical revolution" and renowned for his discovery of the role of oxygen in combustion, was also a pioneer in breath testing.

In 1784 he and Pierre Simon Laplace, the French mathematical physicist, analyzed the breath of a guinea pig. They found that the animal consumed oxygen and expired carbon dioxide. This finding was the first direct evidence that food undergoes combustion in the body. In this discovery lies both the foundation of modern biochemistry and the expression "to be a guinea pig," as Lavoisier confirmed the finding by using himself as a subject.

Lavoisier's apparatus included an ingenious innovation that researchers today still use in various guises: the breath trap. This device accumulates and concentrates components of the breath. Lavoisier's trap was a chemical solution through which he bubbled a large volume of breath. The carbon dioxide in the breath reacted with the solution to form a visible precipitate.

Carbon dioxide is relatively easy to detect, because it makes up approximately 5 percent of the breath. Unfortunately, most other volatile compounds in the breath are present in much lower concentrations, in parts per million or less. Their detection had to wait until the mid-19th century, when colorimetric assays were introduced. In such an analysis, an organic compound interacts with reagents in a solution to produce a change in color.

One of the earliest to exploit colorimetric analysis was A. Nebelthau, a physician at the Marburg Polyclinic in Germany. He constructed a device to analyze the breath of patients suffering from diabetes mellitus. When uncontrolled, this condition causes the blood glucose to rise to excessively high levels; as a result, the body generates large quantities of acetone as a major metabolite. When Nebelthau bubbled a patient's breath through the alkaline iodine trap of his apparatus, he observed a rapid and intense change in color. The change demonstrated that unusual amounts of acetone were being excreted through the lungs.

In 1874 the British physician Francis E. Anstie applied colorimetric analysis to study the fate of alcohol in the human body. His objective was to resolve the controversy raging between physiologists, who believed the body broke down alcohol as it does food, and temperance activists, who thought the body excreted it unchanged as if it were a foreign substance. Anstie's breath trap contained a solution of chromic acid, which changed from red-brown to green in the presence of ethyl alcohol. In an

BREATH-COLLECTION DEVICE in the author's laboratory provides the patient with air purified by activated carbon (in containers at the left). Exhaled breath passes through a drying agent (in container at the right) and is trapped for later analysis. The nose clip ensures the patient breathes through the tubing.

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elegant series of experiments, he demonstrated that the amount of alcohol excreted via the breath and other routes fell far short of the amount consumed. Therefore, most of the alcohol must have been metabolized. (He is also remembered as the originator of Anstie's limit: more than two alcoholic drinks a day may be injurious to one's health, a concept strongly supported by modern findings.)

Current hand-held breath analyzers for alcohol are electronic: the donor blows into a plastic tube, and a spring-loaded piston withdraws approximately one cubic centimeter of breath, which is oxidized in a fuel cell. The resulting electric current is proportional to the concentration of alcohol in the breath. After a few seconds, the breath analyzer calculates and then displays digitally the blood alcohol concentration.

As testing for alcohol demonstrates, it is easy to detect a volatile organic compound in the breath if the substance has been consumed in large quantities beforehand. Consequently, many breath tests require a patient to consume a dose of a specific precursor to a volatile chemical. The

disease reveals itself when an abnormal quantity of the breakdown products appears in the breath.

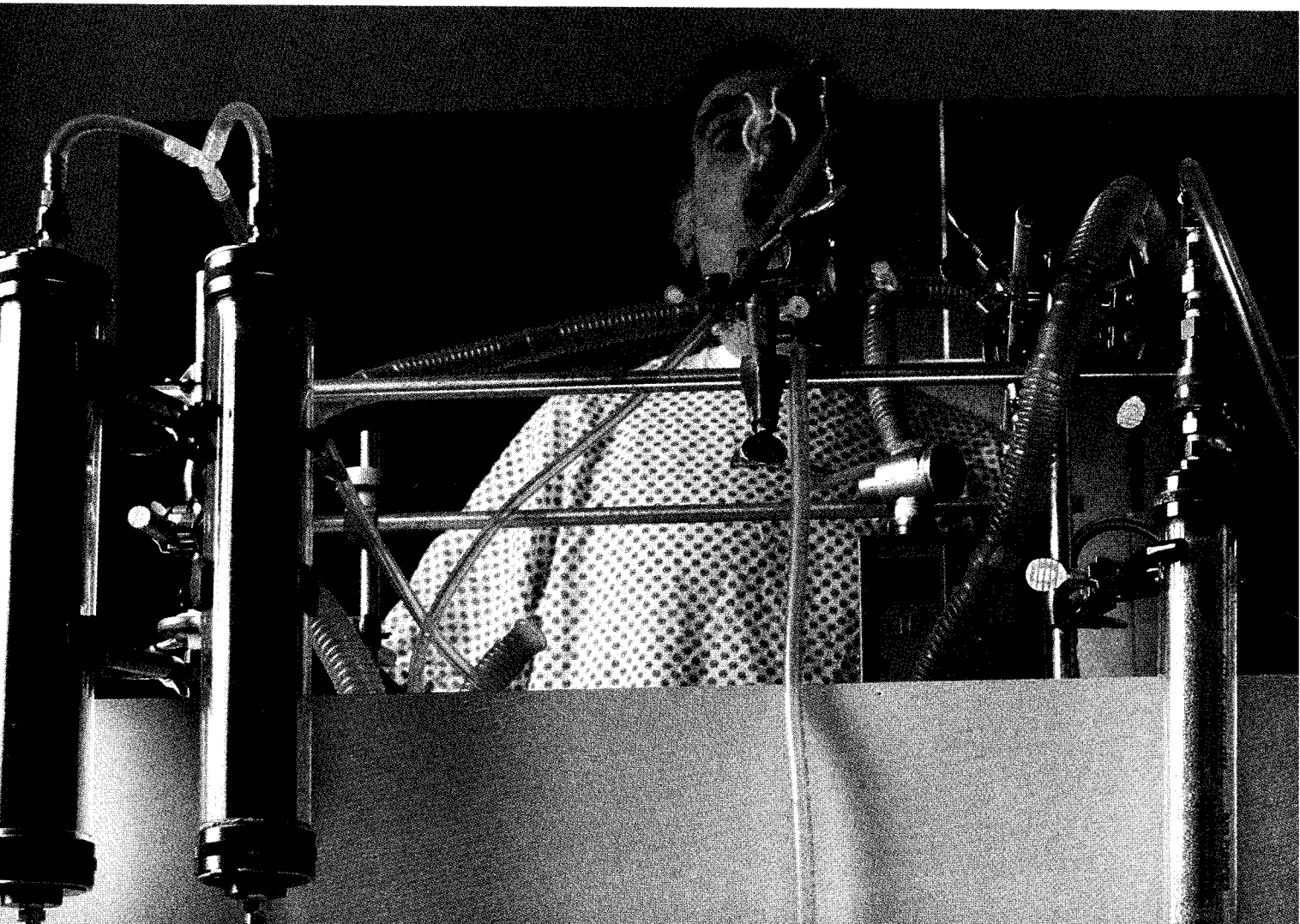
For example, physicians use this approach to diagnose an intestinal disorder known as malabsorption syndrome. This condition causes severe and chronic diarrhea because the diseased intestine cannot absorb food efficiently. One consequence of the syndrome is that most of the sugar in food arrives at the colon intact, where bacteria break it down. The bacterial action releases hydrogen, which is absorbed into the blood and excreted through the lungs. As a test, a physician gives the patient an oral dose of xylose, a five-carbon sugar normally absorbed completely by the small intestine. The appearance of large amounts of hydrogen in the breath within the next few hours confirms the diagnosis of malabsorption syndrome.

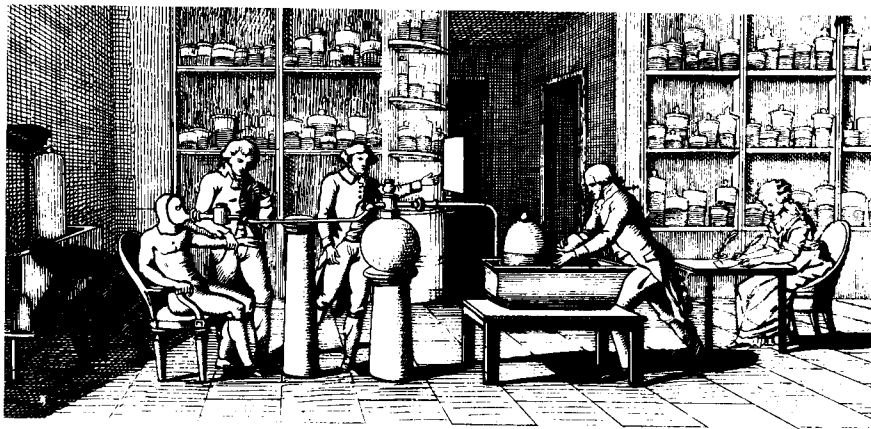
This test can also be refined to demonstrate less common kinds of malabsorption, in which the intestine fails to absorb a specific carbohydrate. For instance, a patient who has a deficiency of lactase in the small bowel will produce hydrogen after a dose of lactose but not after a dose of a different sugar, such as xylose or glucose. Other disorders of the

small intestine, such as bacterial overgrowth, can also be diagnosed by breath testing. This condition may result from any cause of stagnation in the small intestine, including scarring resulting from a surgical procedure or damage to the nerves that control the propulsion rate of food through the gut.

Breath testing is also used to identify damage to the pancreas. Jay A. Perman of the Johns Hopkins University School of Medicine studied children with cystic fibrosis, an often fatal condition that severely harms the pancreas and lungs. He gave them a dose of rice starch, a complex carbohydrate. Children with impaired pancreatic function could not secrete sufficient amylase to digest the meal in the small intestine. Instead the bacteria in the colon completed the digestive task, producing hydrogen, which was detectable in the breath.

Although the presence of hydrogen can help diagnose several disorders of the intestine, other markers are also useful. For example, radioactive carbon 14 is used to identify diseases of the pancreas. The carbon 14 is combined with other compounds that are metabolized by pancreatic enzymes. The amount of radioactive carbon dioxide





LAVOISIER'S LABORATORY to study human breath may be the earliest recorded. In this woodcut, a subject breathes oxygen through a tube, so that the French chemist can study respiration. Madame Lavoisier, seated at the right, records the data.

($^{14}\text{CO}_2$) that subsequently appears in the breath indicates how well the pancreas is functioning. For instance, a diseased pancreas may not secrete sufficient lipase to break down dietary fats. A physician can identify the disorder if a reduced amount of $^{14}\text{CO}_2$ appears in the breath after the patient has consumed a dose of a radiolabeled triglyceride, a low-molecular-weight fat.

Although breath tests for disorders of the small intestine and pancreas are the primary ones now used by the medical community, recent studies have shown that the technique holds promise in recognizing other conditions. Breath tests employing radioactive carbon 14

labeling may be especially useful in diagnosing two of the most common stomach disorders seen in general medical practice: peptic ulcer disease and chronic gastritis. The conditions may result from *Helicobacter pylori* infections, which appear to be quite common. The infections are probably underdiagnosed because confirmation usually requires endoscopy, in which the physician passes an instrument into the stomach to collect a sample of the mucosal lining.

Barry J. Marshall of the University of Virginia Health Sciences Center has shown that breath tests are a highly reliable noninvasive alternative to en-

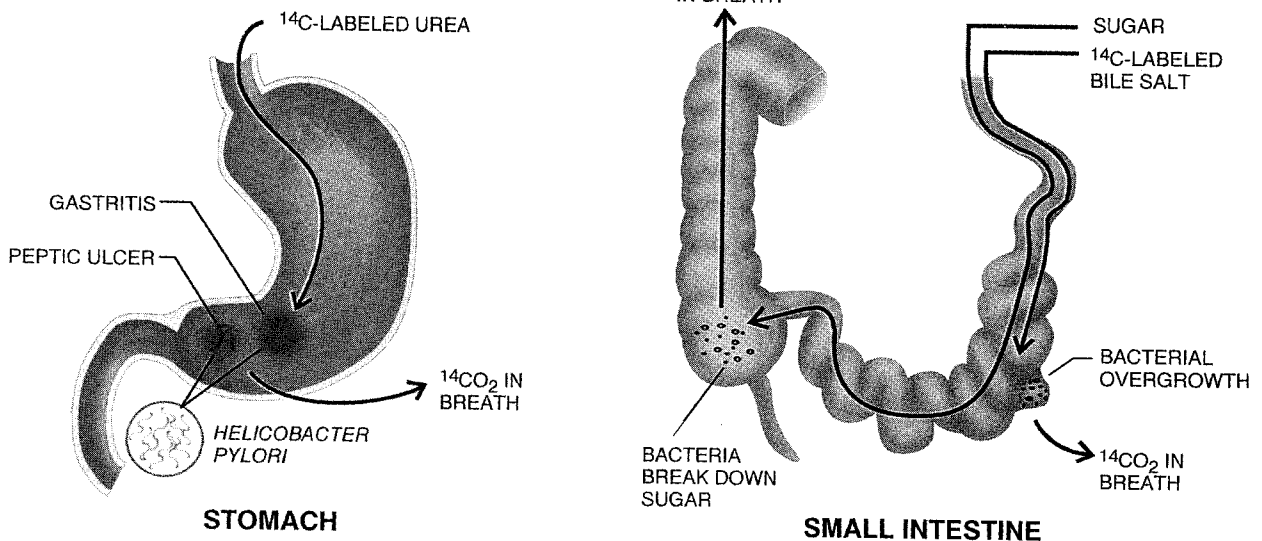
doscopy in the detection of *Helicobacter* infections. *Helicobacter* possesses an enzyme called urease, which is not present in humans. If an infected patient consumes a dose of urea labeled with carbon 14, the urease in *Helicobacter* will break down the urea in the stomach. One of the metabolites will be $^{14}\text{CO}_2$, which is absorbed by the blood and excreted in the breath.

A similar test is also proving useful in uncovering damage to the liver. Conditions such as cirrhosis and hepatitis often go unnoticed in their early stages. By the time bilirubin, a major metabolite of hemoglobin, builds up in the blood to cause the characteristic jaundice, a patient may have lost more than 50 percent of the cells in the liver.

Breath testing can measure the impairment of specific metabolic pathways before jaundice sets in. Specifically, it can test the *N*-demethylation pathway in the hepatic microsomes, which is affected early by any kind of liver damage. The demethylation process in a healthy liver releases substantial amounts of carbon dioxide. To detect liver injury, a physician can give a patient a dose of a tracer compound, such as aminopyrine, phenacetin or galactose, labeled with radioactive carbon 14. Any impairment of *N*-demethylation capacity will reveal itself through a marked reduction in the rate at which $^{14}\text{CO}_2$ appears in the breath.

There are other markers of liver disease. For example, the liver normally

Breath Tests in Gastroenterology



Peptic ulcers and chronic gastritis may result from infections of *Helicobacter pylori*. The bacteria will break down a dose of ^{14}C -labeled urea into $^{14}\text{CO}_2$, which is absorbed by the blood and will appear in the breath.

In malabsorption syndrome, the small intestine is unable to absorb a sugar, such as xylose, lactose, sucrose, glucose, fructose or lactose. Hydrogen will appear in the breath. A dose of a bile salt labeled with carbon 14 will reveal bacterial overgrowth.

breaks down dimethyl sulfide, a volatile metabolite of the amino acid methionine. But if the liver's functions are impaired, a greater quantity of dimethyl sulfide "spills over" into the breath.

Diagnosis of disease is only one realm in which breath testing can be useful. Drug monitoring is a neglected but potentially fruitful field, because most prescribed and illicit drugs have low molecular weights. Their vapor pressures at body temperature may be sufficiently high so that they or their metabolites are propelled into the alveolar air in measurable quantities. Indeed, breath tests for alcohol are so widely employed that it is curious that testing for other drugs is still comparatively unexplored. Metabolites of marijuana can be readily measured in the breath for several days after use.

Breath testing can also illuminate differences in metabolism. It had long been observed that Asians react to the consumption of alcohol in a different manner from most whites. Many Asians experience flushing after a drink, as well as more severe reactions, such as palpitations, nausea and malaise. Breath tests have confirmed that concentrations of acetaldehyde, a volatile metabolite of alcohol, are considerably higher in Asians than in whites after consumption of an alcoholic beverage. Further research has shown that more than 80 percent of Asians generally have relatively low levels of aldehyde dehydrogenase in the liver microsomes. A dose of alcohol re-

sults in a greater buildup of acetaldehyde in the blood, causing flushing and other unpleasant symptoms.

Breath testing is already proving useful in monitoring the exposure of industrial workers to potentially hazardous solvents and petrochemicals. Several of these toxic compounds can be readily measured in human breath. Sensitive breath tests developed by Edo D. Pellizzari, now at the Research Triangle Institute in North Carolina, and his colleagues at the Environmental Protection Agency have shown that toxic chemicals can pollute air far from the factory floor. It is now clear that indoor air in the workplace, office and home may be polluted with small amounts of several compounds, some of them carcinogens. In one study Pellizzari and his colleagues found that workers in hardware stores had ingested volatile compounds that may have originated from carpet solvents and plastics.

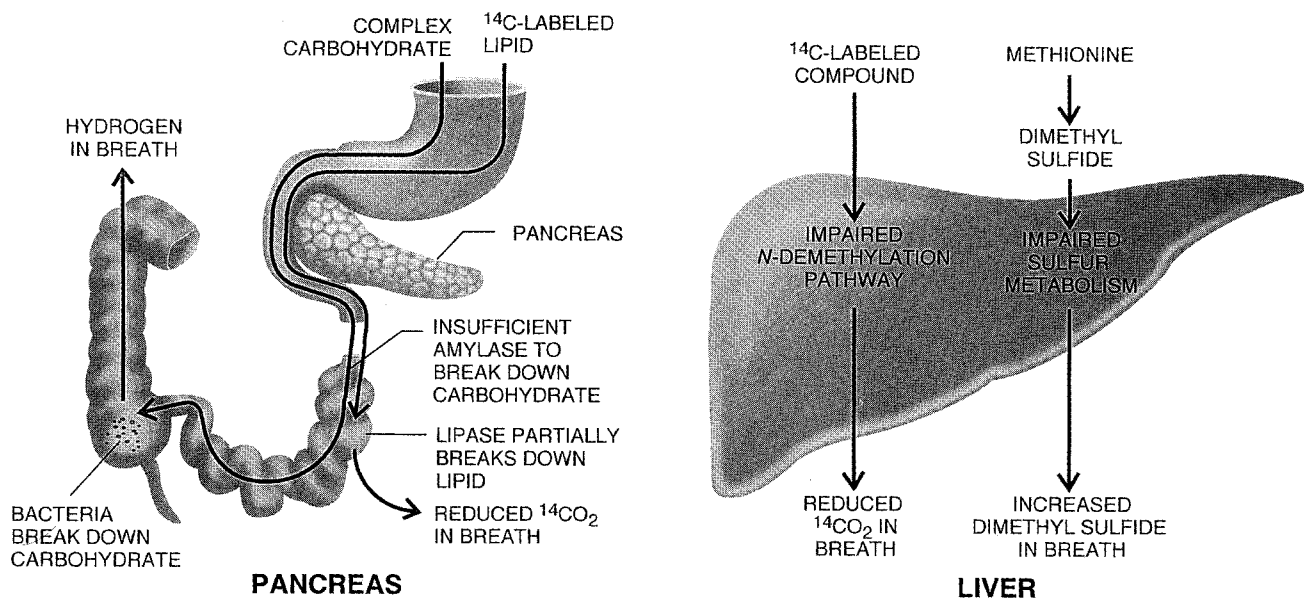
All the breath tests described so far detect either a volatile organic compound that had been previously consumed (such as alcohol) or the metabolites of a precursor. Researchers have attempted to advance one step further by detecting the volatile compounds present in the breath under normal circumstances. In 1971 Linus Pauling described an elegantly simple method for microanalysis of normal breath. He first passed breath through a cold trap consisting of a stainless-steel

tube chilled by dry ice. He then assayed the condensate by gas chromatography and mass spectroscopy. He observed approximately 250 different compounds. This surprisingly large number indicated that the composition of human breath was more complex than had been previously suspected. Other laboratories have confirmed his findings, and researchers have now isolated nearly 400 volatile organic compounds in normal human breath.

Why have scientists not routinely assayed these compounds, if the technology has been available for more than 20 years? Let me attempt to answer this question by inviting the reader to accompany me on an imaginary visit to my laboratory at Bayley Seton Hospital in Staten Island. As you enter, I point out the instruments we use to analyze volatile compounds in breath: thermal desorbers, gas chromatographs and a mass spectrometer, their functions all orchestrated by microcomputers.

But, you wonder, how does one actually collect breath samples in the first place? I now display our collecting apparatus, which is something of an anticlimax. It is a handcrafted and somewhat bizarre-looking device on a wheeled cart. Several meters of plastic tubing wind in seemingly disparate directions; a plywood container conceals a pump and even more tubing. The unit contrasts oddly with the polished elegance of the analytic instruments.

This clash of technological styles is



A lipid labeled with carbon 14 will be broken down by lipase, so any deficiency will result in a below normal amount of ¹⁴CO₂ in the breath. Insufficient production of amylase, which breaks down carbohydrates, will be revealed by the presence of hydrogen.

Impaired functioning of the N-demethylation pathway can be detected by giving aminopyrine, phenacetin or galactose labeled with carbon 14. Sulfur metabolism can be gauged by methionine, which is broken down by the body into dimethyl sulfide.

often encountered in laboratories where breath compounds are studied. The analytic instruments are purchased "off the shelf," while the collection technology consists of the unstandardized gadgetry created by individual tinkerers. Despite two centuries of experience gained since Lavoisier's time, there is still no general agreement on the best way to trap a sample of concentrated breath.

Much of the debate is caused by several difficulties in designing breath-collection equipment. First and foremost, a patient should have no trouble exhaling into the device. Blowing through a liquid trap can be hard work, which probably explains why Lavoisier's subjects stripped to the waist before using the instrument. Even Pauling's device, which included a tube five feet long and 0.2 inch in diameter, could not have been especially easy to blow through. In addition, mouthpiece parts must be replaceable to avoid any risk of transmitting infections from patient to patient.

Contamination of the sample is also a major consideration. Even the cleanest room is polluted with volatile chemicals from such sources as ventilation units and solvents in carpeting adhesive. The trace compounds are measurable as background "noise." In addition, the moisture in breath will condense into droplets on the tubing, which may

contaminate the chemically clean trap.

For meaningful results, the apparatus must be able to sample breath that has originated deep in the lungs, because human breath is not a homogeneous gas. The first 150 milliliters of every expiration consists of "dead space" air from the upper airways, where no gas exchange has occurred (a healthy individual exhales half a liter or more with each breath). The trap should also capture only the volatile compounds of interest and allow the remainder, including nitrogen and oxygen (which together constitute more than 90 percent of the breath), to pass unimpeded.

Generally speaking, there are three types of breath traps: chemical, cryogenic and adsorptive. None is perfect. Chemical trapping usually uses traditional "wet chemistry": breath is bubbled through a reagent solution that captures a specific compound, such as ethanol or acetone. The method is simple and direct, and the trapped derivative, often colored, can be measured easily. The disadvantages are poor sensitivity and the physical effort required of the donor.

In cryogenic trapping, the volatile compounds are captured by freezing. The breath travels through a tube immersed in such cooling fluids as liquid

nitrogen, which is at -196 degrees Celsius. Unfortunately, a cold trap also freezes the water and carbon dioxide in the breath and may rapidly become plugged by ice.

Adsorptive trapping has become the most convenient and most widely used method today. It captures volatile compounds by binding them to agents such as activated carbon and adsorptive resins. Recent advances in microprocessor-controlled thermal desorbers, which automatically "bake off" and concentrate trapped compounds, have made the technique even more convenient.

Several investigators have developed highly sensitive assays using adsorptive traps. They include Pellizzari, M. Sydney Gordon of Battelle, Jack O'Neill of the IIT Research Institute in Chicago and E. S. Kovaleva and R. Liedeman of the All-Union Mental Health Research Center in Moscow.

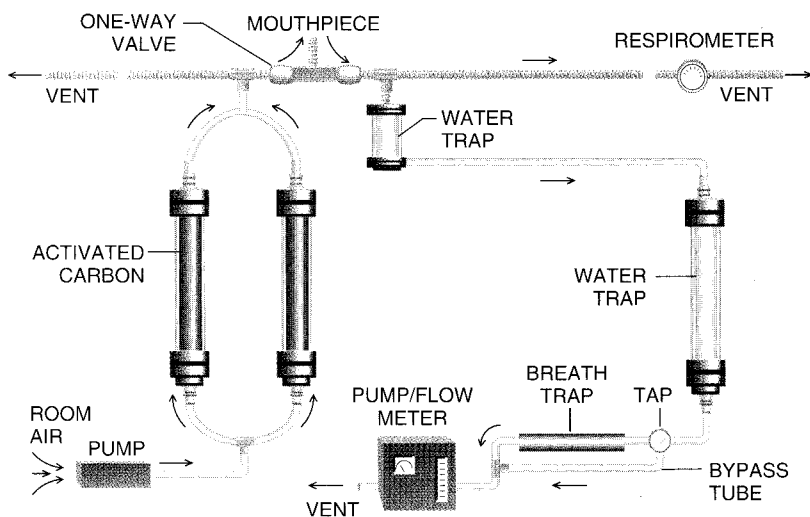
The apparatus that my colleague Joel Greenberg and I have made illustrates how adsorptive trapping can overcome most of the technical difficulties [see illustration on this page]. In our device the donor inhales purified air and breathes out into an exhaust tube. One-way valves ensure that there is no reflux of breath into the reservoir of purified air. There is virtually no resistance to breathing because the tubing is wide (nearly three centimeters in diameter), and the one-way valves require very little pressure to open and close. Even severely ill patients can donate a sample without much discomfort.

No sample is collected from the first part of the breath, which contains the dead-space air. Only alveolar breath from deep in the lungs is extracted from the exhaust tube. Five minutes of collection yields 10 liters of alveolar breath. The breath is desiccated in water traps and drawn through a stainless-steel tube, where activated carbon and an adsorptive resin capture the volatile organic compounds. Later, a thermal desorber automatically strips the volatile compounds from the trap and concentrates them more than 200,000 times, for separation by gas chromatography and assay by mass spectrometry.

The main drawback of adsorptive trapping is that it may have a greater affinity for some compounds than for others. The gas chromatograph is also a source of variation. It separates samples in a long, narrow tube (called the separating column), whose chemical composition influences the number of detectable compounds. Thus, our method yields only about 50 to 60 compounds. This comparatively small number has proved more than sufficient for research purposes.

Collecting Human Breath

This device, in the author's laboratory, is mounted on a cart, which can be wheeled to a patient's bedside [see illustration on page 75]. Room air is pumped through the activated carbon, supplying the volunteer with chemically clean air. Exhaled alveolar breath, drawn through the water trap to remove moisture, passes through the stainless-steel breath trap, which captures the volatile organic compounds. The one-way valves prevent backflow. The respirometer measures the amount of each exhalation.



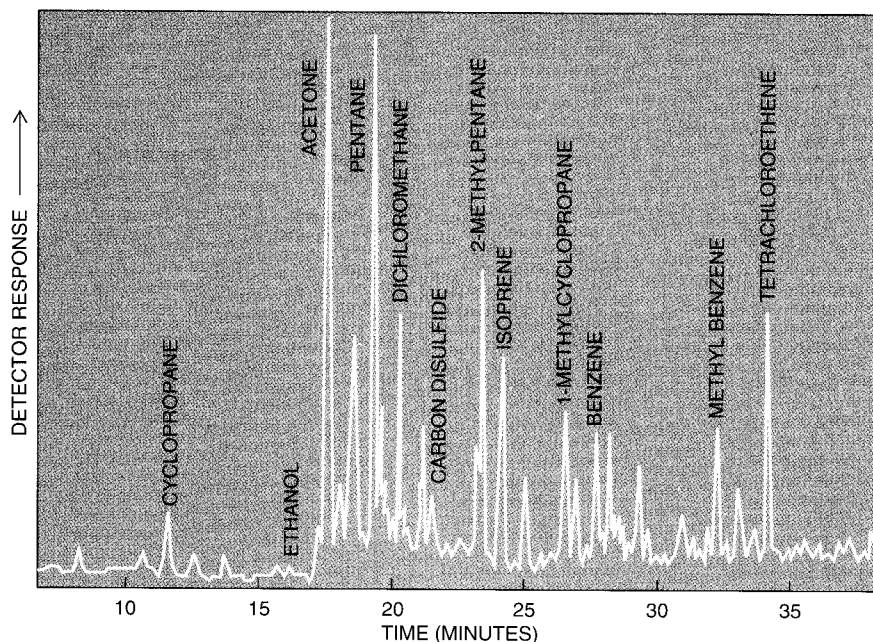
At our current state of knowledge, microanalysis of volatile compounds in the breath has raised more questions than it has answered. The first and most pressing question is, from where do the compounds come? The origin of most is still unknown. Some may be environmental pollutants. For instance, we found carbon disulfide in the breath of all our human volunteers. The compound might be a breakdown product of sulfur-containing amino acids. On the other hand, we have also found carbon disulfide in air samples collected in and around New York City. The origin of the compound is not a trivial concern, because studies have shown that environmental exposure to carbon disulfide can accelerate atherosclerosis and coronary artery disease.

Another problem is the sheer overwhelming mass of data. It is difficult to identify a disease marker among nearly 400 different compounds. Even when abnormal quantities of a compound are observed in patients suffering from a particular illness, physicians must still use rigorous statistical analysis before they can be confident that the findings are not due to chance.

Studies by Gordon and O'Neill have illustrated both the promise and the potential pitfalls of identifying new disease markers in the breath. They assayed volatile chemicals in the breath of patients with lung cancer and found higher than normal concentrations of acetone, methylethylketone, *n*-propanol, tolualdehyde and oxepanone. These findings are exciting, because a breath test for the early detection of lung cancer could become an important new tool in medical practice. But physicians will need more "hard" clinical information about such tests, including its sensitivity and specificity and its positive predictive value. Until then, breath testing for lung cancer will remain only a provocative possibility.

Lack of information now limits the clinical value of most breath tests. Only some are accepted as diagnostic tools. Yet all are fascinating signposts to possible future developments. For example, the measurement of dimethylamine and volatile fatty acids in the breath might enable physicians to recognize diseases of the kidneys and liver in their earliest stages, before any symptoms have developed, without invasive procedures or the administration of a precursor.

Breath tests also offer intriguing clues to the biochemical basis of many diseases whose causes are still unknown. In particular, two alkanes in the breath, pentane and ethane, are known to be elevated in several conditions. Edward J. Zarling of Loyola University Medical



VOLATILE ORGANIC COMPOUNDS in human breath are identified by mass spectroscopy; they show up as large peaks. Only some of the 50 to 60 chemicals detected are labeled. Other assays have discerned nearly 400 compounds.

Center has found increased levels of pentane in the breath of patients suffering from acute myocardial infarction, arthritis or multiple sclerosis. Kovaleva and Liedman discovered that acutely psychotic schizophrenic patients had high concentrations of pentane, which rapidly returned to normal as their clinical condition improved during treatment. Elevated levels of ethane have also been observed in the breath of humans and animals deficient in vitamin E and such trace metals as selenium and copper.

The excretion of pentane and ethane in the breath appears to result from an accumulation of free radicals of oxygen in a damaged cell. These free radicals attack the fatty acids in cell membranes, a process that releases the alkanes. The toxic effects of the free radicals have assumed an increasing importance in recent years, because they seem to mediate the final stages of cellular damage arising from a wide range of causes.

How can breath testing make the transition from the realm of research into the mainstream of clinical practice? The two most pressing needs are for simplification and clinical evaluation. Simplification is essential, because the methods currently employed to collect, concentrate and assay breath samples are too complicated and expensive for general use.

This situation may change during the next decade. New assay instruments on the horizon, such as spectroscopes that

use infrared lasers and mass spectrometers that provide instantaneous readings, may be able to detect low levels of volatile organic compounds in an unconcentrated sample of breath. Improved collection procedures combined with rapid assays should enable researchers to conduct detailed clinical studies of the diagnostic value of breath tests. Physicians and patients of the 21st century may eventually come to think of a breath test in much the same way we now think of a chest x-ray: as an inexpensive and convenient screening test that can help the physician diagnose several diseases in their earliest and most treatable stages.

FURTHER READING

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