Breathtaking – the Sherlock Holmes of gastroenterology

A breathalyzer that catches bacteria in the act

Walter Fabinski, Thomas Weyrauch

In December 2005, the Nobel Prize for Physiology and Medicine was awarded to Barry Marshall and Robin Warren for the discovery of the bacterium *Helicobacter pylori* and its significance in gastritis and gastric ulcers. Their discovery led to a sea change in the treatment of gastric disease, but diagnosis remained reliant on uncomfortable gastroscopy procedures or the use of expensive mass spectrophotometers. A simple, cost-effective solution to this problem was developed by Hartmann and Braun, which is now part of ABB. The new device, based on infrared spectral analysis, is now in use throughout the world.

Marshall and Warren discovered that *H. pylori* lodges in the walls of the stomach with its flagella and can trigger widespread diseases such as gastritis, ulcers and, in the event of chronic infection, cancer. *H. pylori* infection affects roughly half the world's population and is therefore one of the most common bacterial infections. In Germany alone, more than 30 million people are infected with the bacterium, with 5–10 percent going on to develop a gastric or duodenal ulcer. Until Marshall and Warren's discovery, it was believed that no living thing, including bacteria, could survive in the highly acidic environment of the stomach. Patients suffering from ulcers were encouraged to change their lifestyles by avoiding smoking, stress and alcohol, and were treated with histamine blockers. However, this treatment produced only short-term relief and in no way solved the problem. The impact of Marshall and Warren's discovery should not be underestimated. It overturned the fixed worldview in medicine and brought about a paradigm shift. It triggered the search for new methods of diagnosis and treatment through extensive research in a number of scientific fields.

Clarification and concept for diagnosis and treatment

Early research focused on the physiology of the bacterium and its pathogenicity. *H. pylori* 1 is a rod-shaped bacterium that lodges in the protective mucus layer of the stomach wall using its flagella. From this position, it is able to feed and reproduce, while producing toxic substances that further damage the stomach's protective lining. It is this damage that allows corrosive gastric acid to attack the stomach wall, leading to inflammation and more serious complains. It is estimated that practically all duodenal and roughly 80 percent of all gastric ulcers are triggered by *H. pylori*. Chronic inflammation can ultimately lead to cancer. Research revealed that the bacterium is able to convert the urea, which it absorbs as it feeds, into CO_2 and ammonia. The ammonia forms a protective, alkaline coat around the bacterial cell that neutralizes the acidic gastric juices and allows the bacterium to survive. The CO_2 produced by the bacterium is released into the stomach and then escapes via the breathing air. It is this mechanism that betrays the bacterium.

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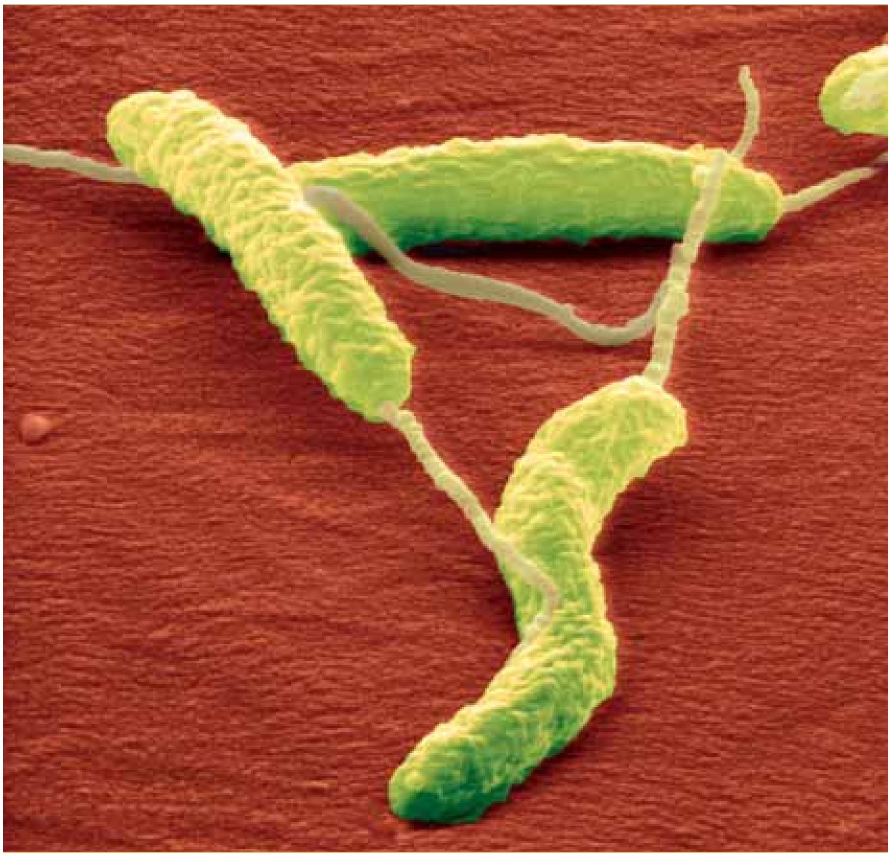
Carbon exists in the form of several naturally occurring isotopes, eg, ^{12}C, ^{13}C, and ^{14}C. Most of the World's carbon is ^{12}C (almost 99 percent), with ^{13}C comprising approximately 1 percent and the rest existing only in very small amounts. In contrast to ^{14}C, which is radioactive, ^{12}C and ^{13}C are stable and completely harmless to man. This means that it is possible to administer an oral dose of ^{13}C-labeled urea to patients suffering from gastritis. After taking the urea, the ratio of $^{13}CO_2$ to $^{12}CO_2$ in the breath of infected patients is altered by the bacterial conversion of ^{13}C-urea into $^{13}CO_2$. Mass spectrometers can be used to determine the ratio of the two isotopes in patients' breath, but these are expensive instruments that are costly to operate and maintain. To solve this problem, a simple, robust, cost-effective, and yet highly sensitive, measuring instrument was required for use in the clinical environment. This was the situation in the early 1990ies when the analytical technology specialist, Hartmann & Braun (which became part of ABB in 1998) was approached.

Development of a diagnosis and treatment concept

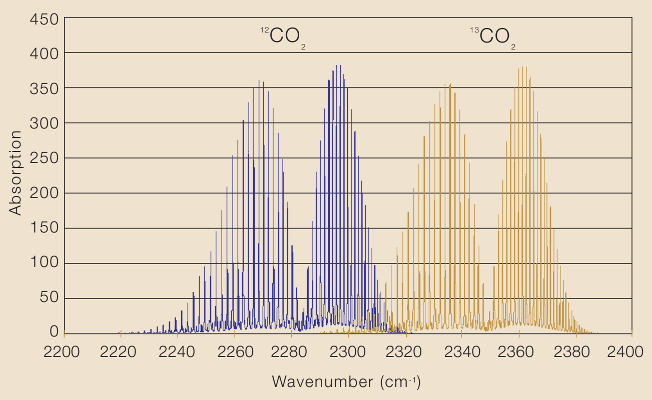
The non-dispersive, infrared, Uras analyzer, which had already proved itself in process-measurement technology, was considered as a potential starting point. The task was to use the Uras measurement technology to

1 View of the *Helicobacter pylori*, taken with a scanning electron microscope



determine the ratio of $^{13}CO_2$ to $^{12}CO_2$ in patients' breath at high resolution, in a drug-compatible system. $^{13}CO_2$ and $^{12}CO_2$ have many common properties, but differ significantly in their atomic masses. This difference is readily detectable by infrared spectral analysis, as shown in 2.

2 Vibration rotational bands of $^{12}CO_2$ and $^{13}CO_2$ in the central infrared range



Because the Uras measurement principle does not require any means of dispersion such as a grating or filters (it uses the gases themselves for selection), results can be compared directly with the unchanging natural spectrum Factbox.

Initial laboratory tests were encouraging and, together with the University of Düsseldorf (Institute for Laser Medicine), Hartmann & Braun developed an adapted Uras instrument for use in the clinical environment. Several specialized manufacturers of medical technology later became involved and took over the global distribution of OEM (original equipment manufacturer) modules from ABB Analytical. The result of the work was a technical appliance capable of comparing breath tests in two steps. The patient simply breathes into the first respiratory bag (the "zero" breath test) and then takes the ^{13}C-labeled urea. Twenty minutes later, the patient provides a second breath sample by breathing into a second bag. The carbon ratios in the two samples are measured and compared. A significant difference in the ratios between the two samples indicates the presence of *Helicobacter*. Recommended therapy for infected patients is usually a 7-day course of so-called "triple therapy," comprising two different antibiotics and an acid blocker.

Practical design and use

The final technical appliance for use in clinical environments was developed by sales partners. The core, however, is the ABB Uras analyzer. This device has been integrated into a small OEM module that has itself been incorporated into a unit suitable for use in clinics. The required sensitivity was achieved by optimizing both the measurement and system technologies. As a result, relatively little substrate is required (a dose of only 75 mg ^{13}C is sufficient), which reduces the cost of the tests (as do reusable respiratory bags).

If results indicate an infection, treatment is carried out in combination with regular breath tests to measure the effectiveness of the therapy. 3 compares the results of a patient infected with *H. pylori* with those of an uninfected patient.

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After developing the instrument technology, extensive clinical trials were carried out and appropriate licensing was obtained. Meanwhile, it was recognized that the global distribution of

Factbox Uras measurement principle

The measurement principle of the Uras gas analyzer is based on the capability of gas molecules to specifically absorb infrared (IR) radiation. This means that energy is removed from a light beam within a certain frequency range, depending on the gas constituents and their concentration, as well as on the length of the absorption cell. In most infrared gas analyzers, a photo detector is used to detect this effect. This is not the case with the Uras analyzer.

The Uras analyzer contains gas-filled, so called, "optopneumatic" detectors, in which the sample of interest is held. The radiant energy absorbed by the sample gas causes a change in temperature and a consequent change in pressure. This pressure change has a magnitude of some nanobars, which is sufficient to evoke an electrical signal via a membrane capacitor. The comparison between the detector gas and the sample gas is highly sensitive to components such as CO, CO_2, SO_2, NO, CH_4, and N_2O – to name but a few.

In the Uras26, serial detectors can reliably determine the concentrations of up to four process gas components. The length of the sample cells installed upstream of the detectors determines the provable concentrations, which range from a few parts per billion by volume (ppbv) (<10–5 percent volume) to 100 percent volume.

The Uras26 also has integrated calibration cells, which automatically move into the optical beam path [1]. These supply a reference signal and ensure long-term stability. Maintenance costs are markedly reduced because built-in calibration dispenses with the need for expensive test gas-cylinders.

A stable measurement is provided by periodic modulation of the IR radiation source with an interrupter disk ("chopper wheel") and subsequent frequency- and phase-selective amplification. This type of signal processing is generally referred to as a "lock-in" process [2].

H. pylori is quite variable. In central and northern European countries, the rate of infection is approximately 30 percent, while in Africa and Asia, the rate can be more than 90 percent. The trials also revealed, however, that infection does not necessarily lead to disease. It is estimated that only around 5 percent of those infected with *H. pylori* experience seriously harmful effects.

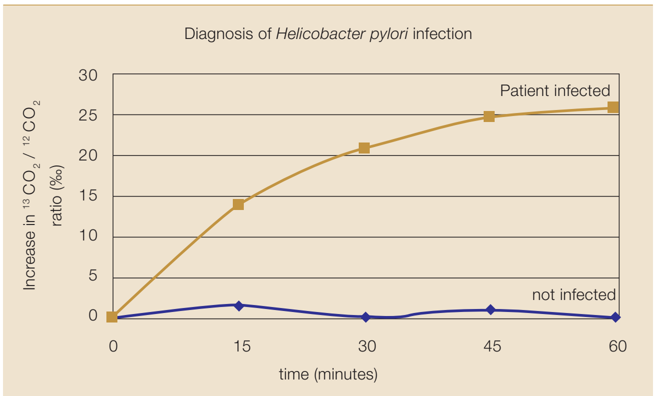
The instrument, sold under the names of Fancy or Iris, has been marketed in industrial countries since the mid-1990ies and is now in use all over the world. In Germany and many other European countries, the costs of the breath tests are borne by health insurance.

ABB is involved in further clinical trials for additional applications. A project is currently examining use of the technology in intensive care units.

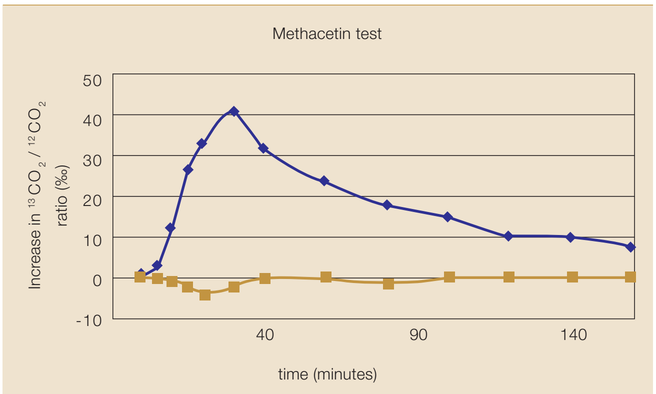
Additional uses

Now that such a convenient method for breath testing has been developed for *H. pylori*, it would seem sensible to adapt it for use in other diagnostic tests. Such efforts are underway for a number of applications, including a liver function test, the assessment of amino acid metabolism, and the tracing of fat malabsorption. These tests rely on ^{13}C-marked substrates that interact only with the organs under scrutiny and on appropriate modifications to the software of the device. These novel applications must also undergo clinical trials and obtain appropriate licensing in accordance with statutory regulations. While gastroscopy clinics are the main market for this application, there are now more than 20 diagnostic tests for other disorders that rely on the accurate and highly-sensitive breath analysis provided by the Uras system. 4 shows an example of liver test. ^{13}C-marked methacetin is administered for this procedure. The upper curve shows the course of $^{13}CO_2/^{12}CO_2$ in a healthy participant. The lower curve shows the course in severe cirrhosis of the liver.

3 Example of a test involving an uninfected patient and one infected with *Helicobacter pylori*. Samples were taken 1 hour after the patients were administered the ^{13}C-labeled urea



4 Example of a liver test



5 The HeliFANplus ^{13}C-infrared analyzer

The instrument is also used worldwide in veterinary medicine and animal nutrition, as well as in biochemical research areas.

The future

The use of the isotope-specific technology described here has great potential in medical diagnosis. In part, this is due to the performance of Uras technology. As a non-dispersive method, it is compact, while providing the required resolution and limit of detection, as well as the necessary robustness for use in a clinical environment. Because it is based on the ratio of two stable isotopes, there is now no need to use radioactive isotopes. ABB is involved in further clinical trials for additional applications. A project is currently examining use of the technology in intensive care units. The German partners are Münster College of Higher Education, the vendors, FAN GmbH with HeliFANplus 5 and WAT GmbH with IRIS, the university clinics in Ulm and Giessen, and the Charité in Berlin. Work is being conducted under the title "Multi-organ function test".

So, if you have a stomach complaint and want to avoid a gastroscopy, ask your doctor for a breath test!

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