MEASUREMENTS OF ETHYLENE CONCENTRATION BY LASER PHOTOACOUSTIC TECHNIQUES WITH APPLICATIONS AT BREATH ANALYSIS

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Abstract. It has been known that the breath contains clues to many diseases. Laser photoacoustic spectroscopy offers a promising new effective technique for the quantitative analysis of trace gases in human breath. This technique enables sensitive, selective detection, quantification and monitoring in real time of gases present in breath. As a typical application, we present precise measurement of trace gases that can be found in the exhaled breath, some of them giving the possibility of a non invasive diagnosis. Exhaled ethylene from many patients was used as a biomarker for lipid peroxidation in lung epithelium following the inhalation of cigarette smoke and X-ray therapy.

Key words: laser photoacoustic spectrometer, breath analysis, ethylene detection, ionising radiation effect.

1. INTRODUCTION

The breath contains valuable information because only a slender barrier separates the air in the alveoli of the lung from the blood capillaries. Specific physiological processes inside human body may be indicated by trace gases originating from the lungs. A physiological process in the body is generally based on many biochemical reactions chained each other and very well regulated by hormones and enzymes. Along a biochemical chained reaction many molecular end products and intermediate products, in excess or discarded, may accumulate in the blood and released trough the pulmonary function in the breath. It is possible to study the time evolution of specific substances found in the breath (markers) to get information about the corresponding physiological processes. The analysis of exhaled breath to detect or asses a given substance with the aim to perform a diagnosis or study a body function is called breath test.

The photoacoustic technique applied to trace gas analysis exploits the infrared absorption characteristics of gases for detection and measurement. This

technique uses strong and modulated sources of infrared radiation to produce heat and sound effects in gases. This sound is then detected by sensitive microphones. During the last years the photoacoustic techniques has been developed to a high degree of perfection.

In this paper we shall describe a laboratory setup used for photoacoustic gas analysis, including the laser source, the spectrophones, the gas handling system and our results on measurement of exhaled ethylene as a biomarker of lipid peroxidation processes.

2. GENERAL SCHEME

The block diagram of the laser photoacoustic spectrometer is presented in Fig. 1. The CW, tunable CO_2-laser beam is chopped, focused by a ZnSe lens, and introduced in the PA cell. After passage through the PA cell, the power of the laser beam is measured by a laser powermeter from Laser Probe Inc. with a measuring head. Its digital output is introduced in the data acquisition interface module together with the output from the lock-in amplifier. All experimental data are processed in real time and stored by a computer.

We have designed, constructed and optimized a rugged sealed-off carbon dioxide laser, step-tunable on more than 60 vibrational-rotational lines and frequency stabilized by the use of plasma tube impedance variations detected as voltage fluctuations. The glass tube has an inner diameter of 7 mm and a discharge
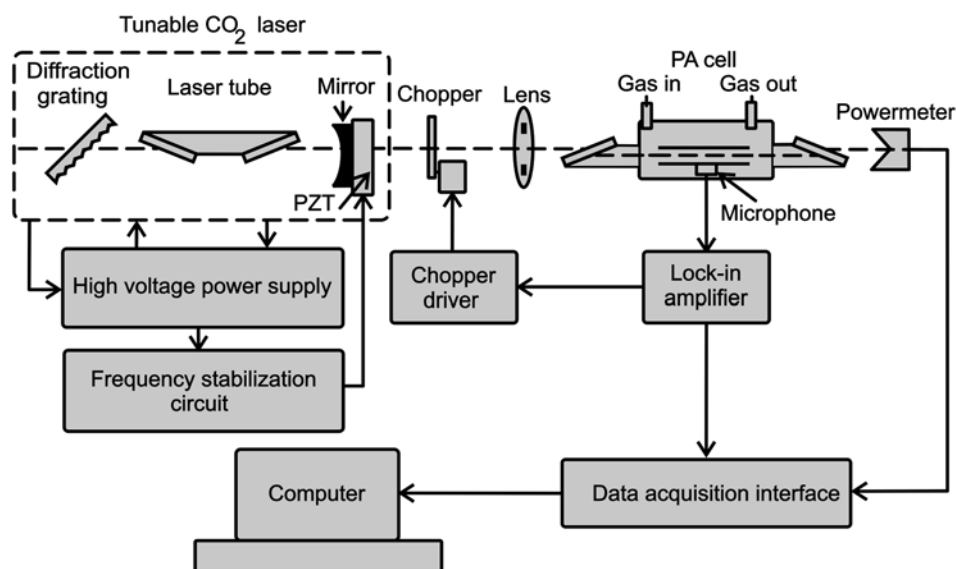

Fig. 1 – Block diagram of the laser photoacoustic spectrometer.

length of 53 mm. At both ends of the tube we attached ZnSe windows at Brewster angle. The laser is water cooled around the discharge tube. The dc discharge is driven by a high-voltage power supply. The end reflectors of the laser cavity are a piezoelectrically driven, partially (85%) reflecting ZnSe mirror at one end and a line-selecting grating at the other. It emits continuous wave (CW) radiation with an output power of 2–7 W and is tunable between 9.2 and 10.8 μm.

The light beam was modulated by a high quality, low vibration noise and variable speed (4–4000 Hz) mechanical chopper model DigiRad C-980 operated at the appropriate resonant frequency of the PA cell (564 Hz).

The PA cell (Fig. 2) is made of stainless steel and Teflon to reduce the outgassing problems and consists of an acoustic resonator (pipe), windows, gas inlets and outlets, and microphones. It also contains at both ends an acoustic filter to suppress the flow and window noise. ZnSe windows at the Brewster angle are glued with epoxy (Torr-Seal) to their respective mounts. The resonant conditions are obtained as longitudinal standing waves in an open tube (resonator) that is placed coaxially inside a larger chamber. We use an open end tube type of resonator, excited in its first longitudinal mode. To achieve a larger signal, we chose a long absorption path length (L = 300 mm) and an inner diameter of the pipe of 7 mm. Gas is admitted and exhausted through two ports located outside the resonator tube. The perturbation of the acoustic resonator amplitude by the air flow noise is thus minimized.
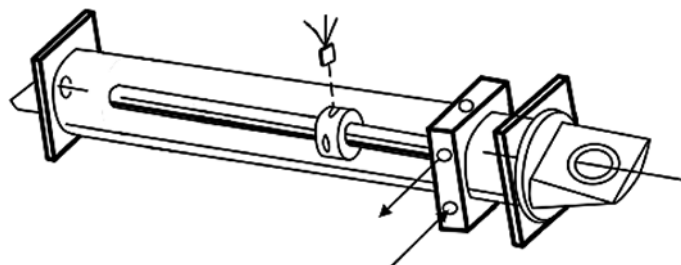

Fig. 2 – Resonant photoacoustic cell.

The generated acoustic waves are detected by microphones mounted in the cell wall; there are four Knowles electrets EK-3033 or EK-23024 miniature microphones in series (sensitivity 20 mV/Pa each at 560 Hz) mounted flush with the wall. They are situated at the loop of the standing wave pattern, at an angle of 90° to one another. The electrical output from these microphones is summed and the signal is selectively amplified by a lock-in amplifier tuned to the chopper frequency. We used a Stanford Research Systems model SR 830 dual phase lock-in amplifier.

Based on a simple model for our system, one can obtain the following formula for the response of the PA system:

$$V = \alpha C P_L S_M c,$$

where: V [V] – photoacoustic signal (peak-to-peak value); α [cm^{-1}atm^{-1}] – gas absorption coefficient at a given wavelength; C [Pa cm W^{-1}] – cell constant; P_L [W] – CW laser power before chopper; S_M [V Pa^{-1}] – microphone responsivity; c [atm] – concentration or partial pressure of the trace gas.

The output signals of the lock-in amplifier and of the powermeter are converted into digital signals by a 12-bit high speed A/D board and processed by a computer.

The acquisition and processing of the recorded data was done with Keithley TestPoint software. TestPoint data acquisition software provides a development environment in which data acquisition applications can be generated. A graphical editor is provided for creating a user interface, or "panel", which the user follows and interacts with as the application executes. TestPoint uses an automated textual description of the operations carried out by each user panel element.

More details on our experimental set up are given in another recent publication [3].

3. GAS HANDLING SYSTEM

The vacuum/gas handling system is an important element in these measurements owing to its role in ensuring gas purity in the PA cell. The Teflon/stainless steel system can perform several functions without necessitating any disconnections. It can be used to pump out the cell, to introduce the sample gas in the PA cell at a controlled flow rate, and monitor the total and partial pressures of gas mixtures.

The schematic of the system is shown in Fig. 3.

We use two gas flow controllers MKS 1179A (0–1000 sccm) and MKS 2259CC (0–200 sccm), which are connected to a digital four-channel instrument MKS 247C, an aluminum-coated plastic bag with sample gas, a potassium hydroxide (KOH) trap, and a cryogenic trap.

To transfer the sample gas from the bag to the PA cell the flow rate was usually set at a low value of 30–100 sccm in all experiments in order to eliminate the acoustic noise of the gas flow; all measurements were carried out with the PA cell at atmospheric pressure.

4. RESULTS AND DISCUSSION

In the present application the photoacoustic spectroscopy is used to reveal traces of ethylene in breath air resulting from lipid peroxidation in lung epithelium
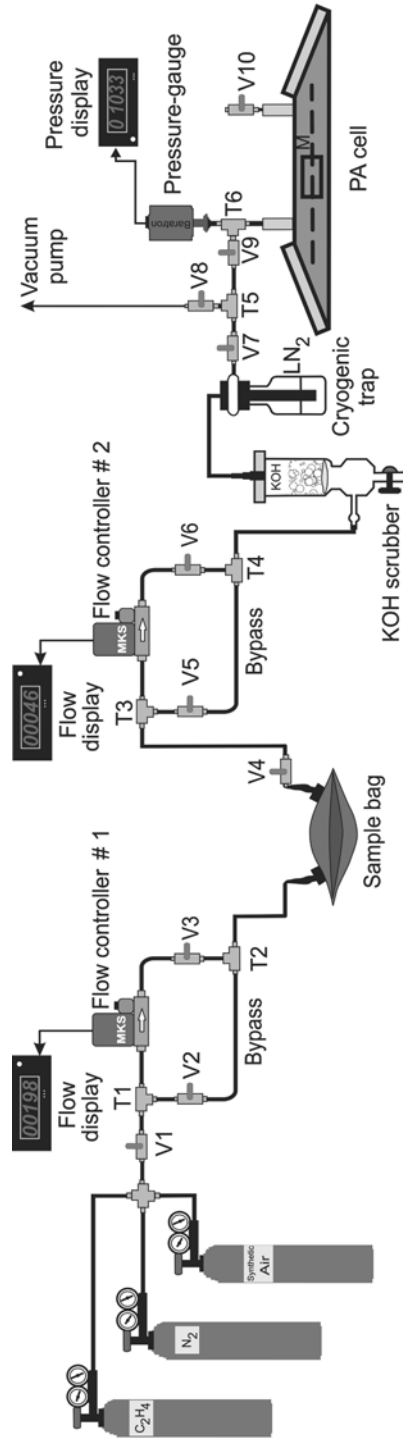
Fig. 3 – The vacuum/gas handling system.

following the inhalation of cigarette smoke and X-ray therapy. Lipid peroxidation is the free-radical-induced oxidative degradation of polyunsaturated fatty acids. Biomembranes and cells are thereby disrupted, causing cell damage and cell death. Lipid peroxidation generates alkenes such as ethane, pentane, which are eliminated in the breath. As a marker of free-radical-mediated damage in the human body, the measurement of the exhaled volatile hydrocarbons, such as ethylene (C_2H_4), is a good noninvasive method to monitor lipid peroxidation.

The exhaled air from the subject being tested is collected inside aluminized bags and then the sample gas is transferred into the measurement PA cell. The breath samples we analyzed were obtained from volunteers who agreed to provide such samples at certain time intervals. The volunteers were asked to exhale into a sample bag with a normal exhalation flow rate. The breath samples were collected in 0.75-liter aluminum-coated bags (Quintron) equipped with valves that sealed them after filling.

The bags were inserted into a modified version of the gas handling system, which ensured better control by means of two independently adjusted flow controllers of the upstream pressure and the flow rate through the sample bag.

A potassium hydroxide (KOH) trap was inserted in the gas circuit to remove the high quantity of CO_2 from the human breath, though is not 100% efficient.

The CO_2 scrubber must neither change the ethylene concentration level, nor introduce new interfering gases. The KOH grains provide an adequate scrubber which also mitigates H_2O interference. Changing the laser line 10P(14) to 10P(16) we remarked the presence of CO_2, due to the fact that the decrease in C_2H_4 concentration was not scaled by the 6 factor (the radiation between the absorption coefficient of the two laser lines). The water vapors are additionally filtered by a cryogenic trap filled with liquid nitrogen.

A. *Trace Of Ethylene In Breath Air Following The Inhalation Of Cigarette Smoke*. The cigarette smoke contains many toxic components (heavy metals, free radicals, chemicals) that may induce ethylene formation by lipid peroxidation in the lung epithelium. Ethylene oxide is a chemical product that induces cancer in the lungs. In order to monitor the damages caused by the inhaled smoke, we performed a breath test which gives us information about the volatile compounds under normal and stress circumstances.

• Nonsmoking person

Human breath contains various volatile organic compounds. The measurement of the exhaled volatile hydrocarbons, such as ethylene, can be altered by CO_2 molecules present in the sample flow.

In order to evaluate the trap efficiency and to establish a reference point for our statistics, we have done the following 3 measurements:

– Without KOH trap we have recorded a value of 1225 ppbV from a ground noise level corresponding to 6 ppbV; this value corresponds to both C_2H_4 and full content of CO_2 in the exhaled air.

– With a small KOH trap with the volume $V = 13$ cm^3 the recorded value decreases at 334.5 ppbV; we still assume the presence of some CO_2 molecules.

– With a larger KOH trap with the volume $V = 88$ cm^3 the value decreases further down to 17.3 ppbV (Fig. 4). According to our calculations in this moment we can presume only the presence of C_2H_4.

So, by using a CO_2 trap with a volume of 88 cm^3 filled with fresh KOH pellets, we succeeded to reduce the CO_2 content in the exhaled breath of a healthy nonsmoking young person.

As a conclusion the level of the CO_2 in the exhaled breath alters considerably the true C_2H_4 concentration and the trap is effective only for a large amount of KOH pellets.

Further discrimination of the CO_2 influence could be done by measurements on two different laser lines: 10P(14) where C_2H_4 shows the maximum absorption and 10P(20) where the absorption coefficient of CO_2 remains nearly the same, while the ethylene one drops by a factor of 17.

• Smoking person

The test for a smoker person in the presence of the large KOH trap shows a high level of ethylene (Fig. 5). At a gas flow rate of 40 sccm a maximum equivalent emission of C_2H_4 measured without trap occurs after 15–16 minutes beeing situated in the range of 1260–1436 ppbV, slightly above the nonsmoker level. When the trap is used, the difference is obvious, the ethylene concentration presenting values at least 10 times higher for a smoker than those registered for the nonsmokers.

B. *Frozen ethylene.* Some authors used also a cryogenic trap based on liquid nitrogen thought to improve the performance/quality of the measurement by retaining the H_2O vapors. Using a simple and small cryogenic trap, we demonstrated the negative influence in our experiment. The liquid nitrogen temperature –196°C (77 K) is bellowing the frozen point of the ethylene gas –169.2°C (104 K) so the practical effects were just to diminish by a factor of 20 the ethylene concentration. Introducing in the flow a calibrated mixture of 1 ppm C_2H_4 in N_2, we observe after filling the cell at 1 atm pressure that the maximum ethylene concentration is only 51 ppb. The level starts to increase suddenly at the point where we stopped the liquid nitrogen admission in the trap (Fig. 6).

In conclusion, a simple nitrogen trap is not suited for our experiments involving ethylene, but a special termocontrolled trap can do the job, setting the working temperature below the sublimation point –78.5°C (194.5 K) freezing point of CO_2 but above –169.2°C (104 K) freezing point of ethylene.

C. *Trace Of Ethylene In Breath Air Following X-ray therapy.* Radiation therapy (also called radiotherapy, X-ray therapy, or irradiation) is the use of certain type of energy (called ionizing radiation) to kill cancer cells and shrink tumors. Radiation therapy injures or destroy cells in the area being treated by damaging their genetic material, making it impossible for these cells to continue grow and divide. Although radiation damages both cancer cells and normal cells, most normal cells can recover from the effects of radiation and function properly. The goal of radiotherapy is to damage as many cancer cells as possible, while limiting harm to nearby healthy tissue.

The effect of ionizing radiation on living cells is supposed to modify the oxidative stress status in the human body through an increase in the peroxidation processes started by the free water radicals generated by indirect radiation effect in living tissue. Important events of the peroxidation take place in the cell membranes determining the release of small linear hydrocarbon molecules through the lipid peroxidation pathways. A fraction of the hydrocarbon molecules generated in the tissue (one among them is the ethylene) will be transported to the lungs by the blood and release in the exhaled breath.

We have analyzed exhaled air from 6 patients between 32 and 77 years old receiving radiation treatment based on X-ray external beam after malign tumor surgery. Breath samples were taken from volunteers at certain time intervals (before, immediately after and at 15 minutes after the X-ray therapy). The patients received fractional doses as high as 2 to 8 Gy depending on the type cancer. For this experiment patients were asked to exhale into sample bags at a normal exhalation flow rate.

To analyze the aluminum bags-contents, firstly we evacuate by the vacuum system the entire gas handling system, including the PA cell, and then we flushed the system with pure nitrogen at atmospheric pressure for 10–20 minutes. For a cell filled with pure the equivalent to a C_2H_4 pressure of ~ 2 ppbV at $P = 5$ W for P(14) laser line.

The exhaled air sample was transferred in the PA cell and analyzed in the continuous nitrogen flux. The potassium hydroxide (KOH) trap inserted in the gas circuit is used to remove as much as possible the high quantity of CO_2 from the exhaled air. To substrate from the final results any influence of the interfering gases (CO_2, H_2O vapors) we applied our changing lines method, using 3 lines: 10P(14), 10P(16), and 10P(26) (Fig. 7).

As reference for our evaluation we have chosen the following values:

– before X-ray therapy: $c = 18.6$ ppbV;
– immediately after X-ray therapy: $c = 23.17$ ppbV;
– 15 min after the X-ray therapy: $c = 10.83$ ppbV.

We have measured the following levels of ethylene for a patient (female, 77 years old) with mammary cancer treated by X-ray therapy with a dose of 8 Gy.

As a first observation of our measurement we see that, indeed, after the X-ray irradiation the ethylene concentration rises showing that lipid peroxidation took place and is possible to detect the process in the very first minute after irradiation The effect of lipid peroxidation is more powerful on the cancer cells, while the healthy cells even affected have higher recovery ability.

A surprising decrease in the level of ethylene concentration was observed in the exhaled air after 15 minutes, the level being even lower than the normal level of the patient (*e.g.* the level measured before any irradiation). This could be explained as a body reaction to the increased level of peroxidic attack: higher the rate of damage, higher the self-defense response of the human organism. Further work is required in order to verify this hypothesis.

5. CONCLUSIONS

In the present work, both the feasibility and the importance of monitoring exhaled ethylene from different patients have been shown. This gas, a biomarker of lipid peroxidation processes, has been measured using a CO_2-laser-based photo-acoustic spectrometer.

The large amount of free radicals contained in cigarette smoke is probably the cause for the lung damage. The test for a smoker person shows a higher level of ethylene compared to nonsmokers and the level of the CO_2 in the expired breath alters considerably the true C_2H_4 concentration and the trap starts to be effective for a large amount of KOH pellets.

Ethylene concentration was also determined in the air exhaled from patients affected by cancer and treated by external radiotherapy. The breath test based on the laser photoacoustic analysis of ethylene might help in monitoring the radiotherapy treatment toxicity.

Measuring human biomarkers in exhaled breath is expected to revolutionize diagnosis and management of many diseases and may soon lead to rapid, improved, lower-cost diagnosis, which will in turn ensure expanded life spans and an improved quality of life.

REFERENCES

1. A. G. Bell, *Upon the production of sound by radiant energy*, Phil. Mag. J. Sci., **XI**, 510–528 (1881).
2. W. H. Preece, *On the conversion of radiant energy into sonorous vibrations*, Proc. R. Soc., **31**, 506–520 (1881).
3. D. C. Dumitras, D. C. Dutu, C. Matei, A. M. Magureanu, C. Popa, *Laser photoacoustic spectroscopy: principles, instrumentation and characterization*, Journal of Optoelectronics and Advanced Materials, **9**, *12*, 3655–3702 (2007).

4. L. B. Kreuzer, *Ultralow gas concentration infrared absorption spectroscopy*, J. Appl. Phys., **42**, 2934–2943 (1971).
5. G. A. West, *Photoacoustic spectroscopy*, Rev. Sci. Instrum., **54**, 797–817 (1983).
6. F. J. M. Harren, G. Cotti, J. Oomens, S. te Lintel Hekkert, *Photoacoustic spectroscopy in trace gas monitoring*, in: *Encyclopedia of Analytical Chemistry*, edited by R. A. Meyers, Vol. **3**, Wiley, Chichester, UK, pp. 2203–2226, 2000.
7. A. Miklos, P. Hess, Z. Bozoki, *Application of acoustic resonators in photoacoustic trace gas analysis and metrology*, Rev. Sci. Instrum., **72**, 1937–1955 (2001).
8. M. W. Sigrist, *Trace gas monitoring by laser photoacoustic spectroscopy and related techniques*, Rev. Sci. Instrum., **74**, 486–490 (2003).
9. A. Puiu, G. Giubileo, C. Bangrazi, *Laser sensors for trace gases in human breath*, Intern. J. Environ. Anal. Chem., **85**, *12–13*, 1001–1012 (2005).
10. Wolfram Miekisch, Jochen K. Schubert, *From highly sophisticated analytical techniques to life-saving diagnostics: Technical developments in breath analysis*, Trends in Analytical Chemistry, **25**, *7* (2006).
11. D. C. Dumitras, D. C. Dutu, N. Comaniciu, V. Draganescu, *Sealed-off CO_2 lasers,* Rev. Roum. Phys., **21**, 559–568 (1976).
12. C.M. Frank Kneepkens, Guy Lepage, Claude C. Roy, *The Potential of the Hydrocarbon Breath Test as a Measure of Lipid Peroxidation*, Free Radical Biology & Medicine, **17**, *2*, pp. 127–160 (1994).
13. A. Karbach, P. Hess, *High precision acoustic spectroscopy by laser excitation of resonant modes*, J. Chem. Phys., **83**, 1075–1084 (1985).
14. J. S. Ryan, M. H. Hubert, R. A. Crane, *Water vapor absorption at isotopic CO_2 laser wavelengths*, Appl. Opt., **22**, 711–717 (1983).
15. F. J. M. Harren, F. G. C. Bijnen, J. Reuss, L. A. C. J. Voesenek, C. W. P. M. Blom, *Sensitive Intercavity Photoacoustic Measurements with a CO_2 Waveguide Laser*, Appl. Phys., **50**, 137–144 (1990).
16. D. C. Dumitras, D.C.A. Dutu, S. Cristescu, C. Mujat, *Laser photoacoustic spectroscopy: A powerful tool for trace gas measurements*, Proc. SPIE, **2778**, 670–671 (1996).
17. G Giubileo, *Medical diagnosis by laser-based analysis of exhaled breath,* Proc. SPIE, **4762**, 318–325 (2002).
18. G. Giubileo, L. DeDominicis, C. C. Lombardi, C. Mancini, A. Antonini, D. C. Dumitras, *Detectability by photoacoustic spectroscopy of X-ray induced ethylene in mice breath,* Proc. SPIE, **5147**, 219–225 (2003).
19. G. Giubileo, A. Puiu, D. C. Dumitras, *Detection of ethylene in smokers breath by laser photoacoustic spectroscopy*, Proc. SPIE, **5486**, 280–286 (2004).
20. D. C. Dumitras, A. Puiu, R. Cernat, G. Giubileo, A. Lai, *Laser photoacoustic spectroscopy: a powerful tool for measurement of trace gases of biological interest at sub-ppb level*, Molecular Crystals and Liquid Crystals Journal, **418**, 217/[945]–227/[955] (2004).
21. D. C. Dumitras, G. Giubileo, A. Puiu, *Investigation of human biomarkers in exhaled breath by laser photoacoustic spectroscopy*, Proc. SPIE, **5850**, 111–121 (2005).
22. T. Fink, S. Büscher, R. Gäbler, Q. Yu, A. Dax, W. Urban, *An improved CO_2 laser intracavity photoacoustic spectrometer for trace gas analysis*, Rev. Sci. Instrum., **67**, 4000–4004 (1996).
23. Michael Phillips, *Breath Tests in Medicine*, Scientific American, 74–79 (1992).