

PART I

BIOMARKERS AND THEIR ROLE IN DRUG DEVELOPMENT

COPYRIGHTED MATERIAL

1

BIOMARKERS ARE NOT NEW

IAN DEWS, MRCP, FFPM

Envestia Ltd., Thame, Oxfordshire, UK

INTRODUCTION

The word *biomarker* in its medical context is a little over 30 years old, having first been used by Karpetsky, Humphrey, and Levy in the April 1977 edition of the *Journal of the National Cancer Institute*, where they reported that the “serum RNase level ... was not a biomarker either for the presence or extent of the plasma cell tumor.” Few new words can have proved so popular—a recent PubMed search lists more than 370,000 publications that use it! Part of this success can no doubt be attributed to the fact that the word gave a long-overdue name to a phenomenon that has been around at least since the seventh century B.C., when Sushutra, the “father of Ayurvedic surgery,” recorded that the urine of patients with diabetes attracted ants because of its sweetness. However, although the origins of biomarkers are indeed ancient, it is fair to point out that the pace of progress over the first 2500 years was somewhat less than frenetic.

UROSCOPY

Because of its easy availability for inspection, urine was for many centuries the focus of attention. The foundation of the “science” of uroscopy is generally attributed to Hippocrates (460–355 B.C.), who hypothesized that urine was a

filtrate of the “humors,” taken from the blood and filtered through the kidneys, a reasonably accurate description. One of his more astute observations was that bubbles on the surface of the urine (now known to be due to proteinuria) were a sign of long-term kidney disease. Galen (A.D. 129–200), the most influential of the ancient Greco-Roman physicians, sought to make uroscopy more specific but in reality added little to the subject beyond the weight of his reputation, which served to hinder further progress in this as in many other areas of medicine.

Five hundred years later, Theophilus Protospatharius, another Greek writer, moved things one step nearer to the modern world when he investigated the effects of heating urine and hence established the world’s first medical laboratory test. He discovered that heating urine from patients with symptoms of kidney disease caused cloudiness (in fact, the precipitation of proteins). In the sixteenth century, Paracelsus (1493–1541) in Switzerland used vinegar to bring out the same cloudiness (acid, like heat, will precipitate proteins).

Events continued to move both farther north and closer to modernity when in 1695 Frederick Deckers of Leiden in the Netherlands identified this cloudiness as resulting from the presence of albumin. The loop was finally closed when Richard Bright (1789–1858), a physician at Guy’s Hospital in London, made the connection between proteinuria and autopsy findings of abnormal kidneys.

The progress from Hippocrates’ bubbles to Bright disease represents the successful side of uroscopy, but other aspects of the subject now strike us as a mixture of common sense and bizarre superstition. The technique of collecting urine was thought to be of paramount importance for accurate interpretation. In the eleventh century, Ismail of Jurjani insisted on a full 24-hour collection in a vessel that was large and clean (very sensible) and shaped like a bladder, so that the urine would not lose its “form” (not at all sensible). His advice to keep the sample out of the sun and away from heat continues, however, to be wise counsel.

Gilles de Corbeil (1165–1213), physician to King Philip Augustus of France, recorded differences in sediment and color of urine which he related to 20 different bodily conditions. He also invented the *matula*, or *jorden*, a glass vessel through which the color, consistency, and clarity of the sample could be assessed. Shaped like a bladder rounded at the bottom and made of thin clear glass, the *matula* was to be held up in the right (not the left) hand for careful inspection against the light. De Corbeil taught that different areas of the body were represented by the urine in different parts of the *matula*. These connections, which became ever more complex, were recorded on uroscopy charts that were published only in Latin, thus ensuring that the knowledge, and its well-rewarded use in treating wealthy patients, was confined to appropriately educated men. To further this education, de Corbeil, in his role as a professor at the Medical School of Salerno, set out his own ideas and those of the ancient Greek and Persian writers in a work called *Poem on the Judgment*

of *Urines*, which was set to music in order that medical students could memorize it more easily. It remained popular for several centuries.

BLOOD PRESSURE

One of the first excursions away from urine in the search for markers of function and disease came in 1555 with the publication of a book called *Sphygmicae artis iam mille ducentos annos perditae & desideratae Libri V* by a physician from Poznań in Poland named Józef Struś (better known by his Latinized name, Iosephus Struthius). In this 366-page work, Struthius described placing increasing weights on the skin over an artery until the pulse was no longer able to lift the load. The weight needed to achieve this gave a crude measure of what he called “the strength of the pulse” or, as we would call it today, blood pressure.

Early attempts at quantitative measurement of blood pressure had to be conducted in animals rather than human subjects because of the invasiveness of the technique. The first recorded success with these techniques dates from 1733, when the Reverend Stephen Hales, a British veterinary surgeon, inserted a brass pipe into a horse’s artery and connected the pipe to a glass tube. Hales observed the blood rising in the tube and concluded not only that the rise was due to the pressure of the blood in the artery but also that the height of the rise was a measure of that pressure.

By 1847, experimental technique had progressed to the point where it was feasible to measure blood pressure in humans, albeit still invasively. Carl Ludwig inserted brass cannulas directly into an artery and connected them via further brass pipework to a U-shaped manometer. An ivory float on the water in the manometer was arranged to move a quill against a rotating drum, and the instrument was known as a *kymograph* (“wave-writer” in Greek).

Meanwhile, in 1834, Jules Hérisson had described his *sphygmomètre*, which consisted of a steel cup containing mercury, covered by a thin membrane, with a calibrated glass tube projecting from it. The membrane was placed over the skin covering an artery and the pressure in the artery could be gauged from the movements of the mercury into the glass tube.

Although minor improvements were suggested by a number of authors over the next few years, credit for the invention of the true *sphygmomanometer* goes to Samuel Siegfried Karl Ritter von Basch, whose original 1881 model used water in both the cuff and the manometer tube. Five years later, Scipione Riva-Rocci introduced an improved version in which an inflatable bag in the cuff was connected to a mercury manometer, but neither of these early machines attracted widespread interest. Only in 1901, when the famous American surgeon Harvey Cushing brought back one of Riva-Rocci’s machines on his return from a trip to Italy, did noninvasive blood pressure measurement really take off.

Sphygmomanometers of the late nineteenth century relied on palpation of the pulse and so could only be used to determine systolic blood pressure. Measurement of diastolic pressure only became possible when Nikolai Korotkoff observed in 1905 that characteristic sounds were made by the constriction of the artery at certain points in the inflation and deflation of the cuff. The greater accuracy allowed by auscultation of these *Korotkoff sounds* opened the way for the massive expansion in blood pressure research that characterized the twentieth century.

IMAGING

To physicians keen to understand the hidden secrets of the human body, few ideas can have been more appealing than the dream of looking through the skin to examine the tissues beneath. The means for achieving this did not appear until a little over a century ago, and then very much by accident. On the evening of November 8, 1895, Wilhem Roentgen, a German physicist working at the University of Würzburg, noticed that light was coming from fluorescent material in his laboratory and worked out that this was the result of radiation escaping from a shielded gas discharge tube with which he was working. He was fascinated by the ability of this radiation to pass through apparently opaque materials and promptly set about investigating its properties in more detail. While conducting experiments with different thicknesses of tinfoil, he noticed that if the rays passed through his hand, they cast a shadow of the bones.

Quick to see the potential medical uses for his new discovery, Roentgen immediately wrote a paper entitled “On a new kind of ray: a preliminary communication” for the Würzburg Physical Medical Society, reprints of which he sent to a number of eminent scientists with whom he was friendly. One of these, Franz Exner of Vienna, was the son of the editor of the *Vienna Presse*, and hence the news was published quickly, first in that paper and then across Europe. Whereas we are inclined to believe that rapid publication is a feature of the Internet age, the Victorians were no slouches in this matter, and by January 24, 1896 a reprint of the Würzburg paper had appeared in the London *Electrician*, a major journal able to bring details of the new invention to a much wider technical audience.

The speed of the response was remarkable. Many physics laboratories already had gas discharge tubes, and within a month physicists in a dozen countries were reproducing Roentgen’s findings. Edwin Frost produced an x-ray image of a patient’s fractured wrist for his physician brother, Gilmon Frost, at Dartmouth College in the United States, while at McGill University in Montreal, John Cox used the new rays to locate a bullet in a gunshot victim’s leg. Similar results were obtained in cities as far apart as Copenhagen, Prague, and Rijeka in Croatia. Inevitably, not everyone was initially quite so impressed; *The Lancet* of February 1, 1896 expressed considerable surprise that the

Belgians had decided to bring x-rays into practical use in hospitals throughout the country! Nevertheless, it was soon clear that a major new diagnostic tool had been presented to the medical world, and there was little surprise when Roentgen received a Nobel Prize in Physics in 1901.

Meanwhile, in March 1896, Henri Becquerel, professor of physics at the Muséum National d'Histoire Naturelle in Paris, while investigating Roentgen's work, wrapped a fluorescent mineral, potassium uranyl sulfate, in photographic plates and black material in preparation for an experiment requiring bright sunlight. However, a period of dull weather intervened, and prior to actually performing the experiment, Becquerel found that the photographic plates were fully exposed. This led him to write: "One must conclude from these experiments that the phosphorescent substance in question emits rays which pass through the opaque paper and reduce silver salts." Becquerel received a Nobel prize, which he shared with Marie and Pierre Curie, in 1903, but it was to be many years before the use of spontaneous radioactivity reached maturity in medical investigation in such applications as isotope scanning and radioimmunoassay.

The use of a fluoroscopic screen on which to view x-ray pictures was implicit in Roentgen's original discovery and soon became part of the routine equipment not only of hospitals but even of shoe shops, where large numbers of children's shoe fittings were carried out in the days before the true dangers of radiation were appreciated. However, the greatest value of the real-time viewing approach only emerged following the introduction of electronic image intensifiers by the Philips company in 1955.

Within months of the introduction of planar x-rays, physicians were asking for a technique that would demonstrate the body in three dimensions. This challenge was taken up by a number of scientists in different countries, but because of the deeply ingrained habit of reviewing only the national, not the international, literature, these workers remained ignorant of each other's progress for many years.

Carl Mayer, a Polish physician, first suggested the idea of tomography in 1914. André-Edmund-Marie Bocage in France, Gustav Grossmann in Germany, and Allesandro Vallebona in Italy all developed the idea further and built their own equipment. George Ziedses des Plantes in the Netherlands pulled all these strands together in the 1930s and is generally considered the founder of conventional tomography.

Further progress had to wait for the development of powerful computers, and it was not until 1972 that Godfrey Hounsfield, an engineer at EMI, designed the first computer-assisted tomographic device, the EMI scanner, installed at Atkinson Morley Hospital, London, an achievement for which he received both a Nobel prize and a knighthood.

Parallel with these advances in x-ray imaging were ongoing attempts to make similar use of the spontaneous radioactivity discovered by Becquerel. In 1925, Herrman Blumgart and Otto Yens made the first use of radioactivity as a biomarker when they used bismuth-214 to determine the arm-to-arm

circulation time in patients. Sodium-24, the first artificially created biomarker radioisotope, was used by Joseph Hamilton to investigate electrolyte metabolism in 1937.

Unlike x-rays, however, radiation from isotopes weak enough to be safe was not powerful enough to create an image merely by letting it fall on a photographic plate. This problem was solved when Hal Anger of the University of California, building on the efficient gamma-ray capture system using large flat crystals of sodium iodide doped with thallium developed by Robert Hofstadter in 1948, constructed the first gamma camera in 1957.

The desire for three-dimensional images that led to tomography with x-rays also influenced radioisotope imaging and drove the development of single-photon-emission computed tomography (SPECT) by David Kuhl and Roy Edwards in 1968. Positron-emission tomography (PET) also builds images by detecting energy given off by decaying radioactive isotopes in the form of positrons that collide with electrons and produce gamma rays that shoot off in nearly opposite directions. The collisions can be located in space by interpreting the paths of the gamma rays, and this information is then converted into a three-dimensional image slice. The first PET camera for human studies was built by Edward Hoffman, Michael Ter-Pogossian, and Michael Phelps in 1973 at Washington University. The first whole-body PET scanner appeared in 1977.

Radiation, whether from x-ray tubes or from radioisotopes, came to be recognized as having dangers both for the patient and for personnel operating the equipment, and efforts were made to discover media that would produce images without these dangers. In the late 1940s, George Ludwig, a junior lieutenant at the Naval Medical Research Institute in Bethesda, Maryland, undertook experiments using industrial ultrasonic flaw-detection equipment in an attempt to determine the acoustic impedance of various tissues, including human gallstones surgically implanted into the gallbladders of dogs. His observations were detailed in a 30-page project report to the Naval Medical Research Institute dated June 16, 1949, now considered the first report of its kind on the diagnostic use of ultrasound. However, a substantial portion of Ludwig's work was considered classified information by the Navy and was not published in medical journals.

Civilian research into what became the two biggest areas of early ultrasonic diagnosis—cardiology and obstetrics—began in Sweden and Scotland, respectively, both making use of gadgetry initially designed for shipbuilding. In 1953, Inge Edler, a cardiologist at Lund University collaborated with Carl Hellmuth Hertz, a graduate student in the department of nuclear physics who was familiar with using ultrasonic reflectoscopes for nondestructive materials testing, and together they developed the idea of using this method in medicine. They made the first successful measurement of heart activity on October 29, 1953 using a device borrowed from Kockums, a Malmö shipyard. On December 16 of the same year, the method was used to generate an echo encephalogram. Edler and Hertz published their findings in 1954.

At around the same time, Ian Donald of the Glasgow Royal Maternity Hospital struck up a relationship with boilermakers Babcock & Wilcox in Renfrew, where he used their industrial ultrasound equipment to conduct experiments assessing the ultrasonic characteristics of various in vitro preparations. With fellow obstetrician John MacVicar and medical physicist Tom Brown, Donald refined the equipment to the point where it could be used successfully on live volunteer patients. These findings were reported in *The Lancet* on June 7, 1958 as “Investigation of abdominal masses by pulsed ultrasound.”

Nuclear magnetic resonance (NMR) in molecules was first described by Isidor Rabi in 1938. His work was followed up eight years later by Felix Bloch and Edward Mills Purcell, who, working independently, noticed that magnetic nuclei such as hydrogen and phosphorus, when placed in a magnetic field of a specific strength, absorb radio-frequency energy, a situation described as being “in resonance.”

For the next 20 years NMR found purely physical applications in chemistry and physics, and it was not until 1971 that Raymond Damadian showed that the nuclear magnetic relaxation times of different tissues, especially tumors, differed, thus raising the possibility of using the technique to detect disease. Magnetic resonance imaging (MRI) was first demonstrated on small test tube samples in 1973 by Paul Lauterbur, and in 1975 Richard Ernst proposed using phase and frequency encoding and the Fourier transform, the technique that still forms the basis of MRI.

The first commercial nuclear magnetic imaging scanner allowing imaging of the body appeared in 1980 using Ernst’s technique, which allowed a single image to be acquired in approximately 5 minutes. By 1986, the imaging time was reduced to about 5 seconds without sacrificing too much image quality. In the same year, the NMR microscope was developed, which allowed approximately 10-mm resolution on approximately 1-cm samples. In 1993, functional MRI (fMRI) was developed, thus permitting the mapping of function in various regions of the brain.

ELECTROCARDIOGRAPHY

Roentgen’s discovery of x-rays grew out of the detailed investigation of electricity that was a core scientific concern of the nineteenth century, and it is little surprise that investigators also took a keen interests in the electricity generated by the human body itself. Foremost among these was Willem Einthoven. Before his day, although it was known that the body produced electrical currents, the technology was inadequate to measure or record them with any sort of accuracy. Starting in 1901, Einthoven, a professor at the University of Leiden, conducted a series of experiments using a string galvanometer. In his device, electric currents picked up from electrodes on the patient’s skin passed through a thin filament running between very

strong electromagnets. The interaction of the electric and magnetic fields caused the filament or “string” to move, and this was detected by using a light to cast a shadow of the moving string onto a moving roll of photographic paper.

It was not, at first, an easy technique. The apparatus weighed 600lb, including the water circulation system essential for cooling the electromagnets, and was operated by a team of five technicians. Over the next two decades Einthoven gradually refined his machine and used it to establish the electrocardiographic (ECG) features of many different heart conditions, work that was eventually recognized with a Nobel prize in 1924.

As the ECG became a routine part of medical investigations it was realized that a system that gave only a “snapshot” of a few seconds of the heart’s activity could be unhelpful or even misleading in the investigation of intermittent conditions such as arrhythmias. This problem was addressed by Norman Holter, an American biophysicist, who created his first suitcase-sized “ambulatory” monitor as early as 1949, but whose technique is dated in many sources to the major paper that he published on the subject in 1957, and other authors cite an even later, 1961 publication.

HEMATOLOGY

The scientific examination of blood in order to learn more about the health of the patient from whom it was taken can be dated to 1642, when Anthony van Leeuwenhoek first observed blood cells through his newly invented microscope. Progress was at first slow, and it was not until 1770 that leucocytes were discovered by William Hewson, an English surgeon, who also observed that red cells were flat rather than spherical, as had earlier been supposed.

Association of blood cell counts with clinical illness depended on the development of a technical method by which blood cells could be counted. In 1852, Karl Vierordt at the University of Tübingen developed such a technique, which, although too tedious for routine use, was used by one of his students, H. Welcher, to count red blood cells in a patient with “chlorosis” (an old word for what is probably our modern iron-deficiency anemia). He found, in 1854, that an anemic patient had significantly fewer red blood cells than did a normal person. Platelets, the third major cellular constituent of blood, were identified in 1862 by a German anatomist, Max Schultze.

Remarkably, all these discoveries were made without the benefit of cell staining, an aid to microscopic visualization that was not introduced until 1877 in Paul Ehrlich’s doctoral dissertation at the University of Leipzig. The movement of blood cell studies from the research laboratory to routine support of patient care needed a fast automatic technique for separating and counting cells, which was eventually provided by the Coulter brothers, Wallace and Joseph. In 1953 they patented a machine that detected the change in electrical conductance of a small aperture as fluid containing cells was drawn through.

Cells, being nonconducting particles, alter the effective cross section of the conductive channel and so signal both their presence and their size.

An alternative technique, flow cytometry, was also developed in stages between the late 1940s and the early 1970s. Frank Gucker at Northwestern University developed a machine for counting bacteria in a laminar stream of air during World War II and used it to test gas masks, the work subsequently being declassified and published in 1947. Louis Kametsky at IBM Laboratories and Mack Fulwyler at the Los Alamos National Laboratory experimented with fluidic switching and electrostatic cell detectors, respectively, and both described cell sorters in 1965. The modern approach of detecting cells stained with fluorescent antibodies was developed in 1972 by Leonard Herzenberg and his team at Stanford University, who coined the term *fluorescence-activated cell sorter* (FACS).

BLOOD AND URINE CHEMISTRY

As with hematology, real progress in measuring the chemical constituents of plasma depended largely on the development of the necessary technology. Until such techniques became available, however, ingenious use was made of bioassays, developed in living organisms or preparations made from them, to detect and in some cases quantify complex molecules. A good example of this is the detection of human chorionic gonadotrophin (hCG) in urine as a test for pregnancy. Selmar Aschheim and Bernhard Zondek in Berlin, who first isolated this hormone in 1928, went on to devise the Aschheim–Zondek pregnancy test, which involved five days of injecting urine from the patient repeatedly into an infantile female mouse which was subsequently killed and dissected. The finding of ovulation in the mouse indicated that the injected urine contained hCG and meant that the patient was pregnant.

In the early 1940s, the mouse test gave way to the frog test, introduced by Lancelot Hogben in England. This was a considerable improvement, in that injection of urine or serum from a pregnant woman into the dorsal lymph sac of the female African clawed frog (*Xenopus laevis*) resulted in ovulation within 4 to 12 hours. Although this test was known to give a relatively high proportion of false negatives, it was regarded as an outstanding step forward in diagnosis. One story from the 1950s recounts that with regard to the possible pregnancy of a particular patient, “opinions were sought from an experienced general practitioner, an eminent gynecologist, and a frog; only the frog proved to be correct.”

Pregnancy testing, and many other “biomarker” activities, subsequently moved from out-and-out bioassays to the “halfway house” of immunological tests based on antibodies to the test compound generated in a convenient species but then used in an *ex vivo* laboratory setting, and in 1960 a hemagglutination inhibition test for pregnancy was developed by Leif Wide and Carl Gemzell in Uppsala.

Not all immune reactions can be made to modulate hemagglutination, and a problem with the development of immunoassays was finding a simple way to detect whether the relevant antibody or antigen was present. One answer lay in the use of radiolabeled reagents. Radioimmunoassay was first described in a paper by Rosalyn Sussman Yalow and Solomon Berson published in 1960.

Radioactivity is difficult to work with because of its safety concerns, so an alternative was sought. This came with the recognition that certain enzymes (such as ABTS or 3,3',5,5'-tetramethylbenzidine) which react with appropriate substrates to give a color change could be linked to an appropriate antibody. This linking process was developed independently by Stratis Avrameas and G. B. Pierce. Since it is necessary to remove any unbound antibody or antigen by washing, the antibody or antigen must be fixed to the surface of the container, a technique first published by Wide and Porath in 1966.

In 1971, Peter Perlmann and Eva Engvall at Stockholm University, as well as Anton Schuurs and Bauke van Weemen in the Netherlands, independently published papers that synthesized this knowledge into methods to perform enzyme-linked immunosorbent assay (ELISA).

A further step toward physical methods was the development of chromatography. The word was coined in 1903 by the Russian botanist Mikhail Tswett to describe his use of a liquid–solid form of a technique to isolate various plant pigments. His work was not widely accepted at first, partly because it was published in Russian and partly because Arthur Stoll and Richard Willstätter, a much better known Swiss–German research team, were unable to repeat the findings.

However, in the late 1930s and early 1940s, Archer Martin and Richard Synge at the Wool Industries Research Association in Leeds devised a form of liquid–liquid chromatography by supporting the stationary phase, in this case water, on silica gel in the form of a packed bed and used it to separate some acetyl amino acids derived from wool. Their 1941 paper included a recommendation that the liquid mobile phase be replaced with a suitable gas that would accelerate the transfer between the two phases and provide more efficient separation: the first mention of the concept of gas chromatography. In fact, their insight went even further, in that they also suggested the use of small particles and high pressures to improve the separation, the starting point for high-performance liquid chromatography (HPLC).

Gas chromatography was the first of these concepts to be taken forward. Erika Cremer working with Fritz Prior in Germany developed gas–solid chromatography, while in the UK, Martin himself cooperated with Anthony James in the early work on gas–liquid chromatography published in 1952. Real progress in HPLC began in 1966 with the work of Csaba Horváth at Yale. The popularity of the technique grew rapidly through the 1970s, so that by 1980, this had become the standard laboratory approach to a wide range of analytes. The continuing problem with liquid or gas chromatography was the identification of the molecule eluting from the system, a facet of the techniques that was to be revolutionized by mass spectrometry.

The foundations of mass spectrometry were laid in the Cavendish Laboratories of Cambridge University in the early years of the twentieth century. Francis Aston built the first fully functional mass spectrometer in 1919 using electrostatic and magnetic fields to separate isotope ions by their masses and focus them onto a photographic plate. By the end of the 1930s, mass spectrometry had become an established technique for the separation of atomic ions by mass.

The early 1950s saw attempts to apply the technique to small organic molecules, but the mass spectrometers of that era were extremely limited by mass and resolution. Positive theoretical steps were taken, however, with the description of time-of-flight (TOF) analysis by W. C. Wiley and I. H. Maclaren, and quadrupole analysis by Wolfgang Pauli.

The next major development was the coupling of gas chromatography to mass spectrometry in 1959 by Roland Gohlke and Fred McLafferty at the Dow Chemical Research Laboratory in Midland, Michigan. This allowed, for the first time, an analysis of mixtures of analytes without laborious separation by hand. This, in turn, was the trigger for the development of modern mass spectrometry of biological molecules.

The introduction of liquid chromatography–mass spectrometry (LC-MS) in the early 1970s, together with new ionization techniques developed over the last 25 years (i.e., fast particle desorption, electrospray ionization, and matrix-assisted laser desorption/ionization), have made it possible to analyze almost every class of biological compound class right up into the megadalton range.

FASHIONABLE “OMICS”

In Benet Street, Cambridge, stands a rather ordinary pub which on Saturday, February 28, 1953, enjoyed 15 minutes of fame far beyond Andy Warhol's wildest dreams. Two young men arrived for lunch and, as James Watson watched, Francis Crick announced to the regulars in the bar that “we have found the secret of life.” The more formal announcement of the structure of DNA appeared in *Nature* on April 2 in a commendably brief paper of two pages with six references. Watson and Crick shared a Nobel prize with Maurice Wilkins, whose work with Rosalind Franklin at King's College, London had laid the groundwork. Sadly, Franklin's early death robbed her of a share of the prize, which is never awarded posthumously.

Over the next two decades a large number of researchers teased out the details of the genetic control of cells, and by 1972 a team at the Laboratory of Molecular Biology of the University of Ghent, led by Walter Fiers, were the first to determine the sequence of a gene (a coat protein from a bacteriophage). The same team followed up in 1976 by publishing the complete RNA nucleotide sequence of the bacteriophage. The first DNA-based genome to be sequenced in its entirety was the 5368-base-pair sequence of bacteriophage

Φ-X174 elucidated by Frederick Sanger in 1977. The science of genomics had been born.

Although the rush to sequence the genomes of ever more complex species (including humans in 2001) initially held out considerable hope of yielding new biomarkers, focus gradually shifted to the protein products of the genes. This process is dated by many to the introduction in 1977 by Patrick O'Farrell at the University of Colorado in Boulder of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). The subject really took off in the 1990s, however, with technical improvements in mass spectrometers combined with computing hardware and software to support the extremely complex analyses involved.

The next “omics” to become fashionable was metabolomics, based on the realization that the quantitative and qualitative pattern of metabolites in body fluids reflects the functional status of an organism. The concept is by no means new, the first paper addressing the idea (but not using the word) having been “Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography” by Robinson and Pauling in 1971. The word *metabolomics*, however, was not coined until the 1990s.

THE FUTURE

Two generalizations may perhaps be drawn from the accelerating history of biomarkers over the last 2700 years. The first is that each new step depends on an interaction between increasing understanding of the biology and technical improvement of the tools leading to a continuous spiral of innovation. The second is the need for an open but cautious mind. Sushustra's recognition of the implications of sweet urine has stood the test of time; de Corbeil's *Poem on the Judgment of Urines* has not. The ultimate fate of more recent biomarkers will only be revealed by time.