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History of Clinical Chemistry

Wöhler & the Birth of Clinical Chemistry[1] Ian Wilkinson, BS, MS, PhD, DClinChem, CSCC(Cert), FCACB, FACB, MBA[2]

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Clinical chemistry is concerned with the analysis of body fluids to yield timely, relevant, accurate and precise information on the clinical status of the human body. From the viewpoint of the clinical chemist, patients are 'black boxes', complex metabolic machines that process molecules to produce energy and to oppose entropy. Considerable time, effort and money are expended in attempting to find out what is happening inside this box. Clinical diagnosis is essentially the interpretation of relevant data obtained from the box, the process of separating signal from noise and then giving the signal meaning. Throughout the world, hundreds of thousands of body fluid specimens are analyzed every day and the data obtained are interpreted and used in assessing the health of patients. This is such a commonplace occurrence that we seldom stop to question it, or consider the implicit assumption that is being made, i.e., that in vivo processes can be understood by analyzing their constituents in vitro. In other words, that data obtained from a body fluid sample can be used to infer information about the status of the living organism from which it came. This assumption is the cornerstone upon which clinical chemistry and related disciplines are based. The conceptual chasm between in vivo processes and in vitro analysis, between life and the test tube, was not bridged until 1828 when Wöhler synthesized urea[1] in the absence of any 'vital force' or living organism.

A vital force In the 19th century, leading physiologists including Marie François Xavier Bichat (1771-1802), Johannes Müller (1801-58) and Justus, Baron von Liebig (1803-73) believed that processes within living organisms were unique and could not be duplicated in the laboratory. Consequently, the in vitro synthesis of 'organic' compounds was believed to be impossible. It was postulated that living organisms contained a 'vital force' that was the very essence of life. This dogma of a 'vital force' pervaded art and science. A 'vital force' (in this case 'galvanic') was required, to bring Frankenstein's monster to life, in Mary Shelley's (1797-1851) proto- science fiction novel written in 1816. Vitalism held that no substance produced by living organisms could be synthesized by combining inanimate chemicals in a lifeless container in the laboratory. To attempt such a synthesis was considered a futile task because of the absence of a 'vital force', an enabling factor present in all living things but absent from inanimate objects[2]. Vitalists believed that life cannot be understood in terms of chemical or physical properties alone. There was a hidden synergy within all living things, which exceeded the sum of their material parts.

When René Joachim Henri Dutrochet (1776-1847) discovered endosmosis, he explained this phenomenon not, as we might expect, in terms of physical forces, but as due to a 'vital physico-organic' force[3]. The spectre of 'vitalism' continued to haunt the biological sciences well into the present century. Sir Arthur Eddington (1882-1944), a leading proponent of Einstein's theory of relativity and the first physicist to confirm through observation of the 1919 total eclipse of the sun, the prediction that curvature of space-time by a massive object would 'bend' nearby light rays from distant stars, therefore appearing to shift their position. Despite his acceptance of Einstein's revolutionary theory of space, time and gravity, Eddington believed firmly that living organisms possessed an unknown force above and beyond those explained by biochemists and physiologists [4].

Descartes, Darwin and the dissenters One of the first to challenge the vitalists' viewpoint was René Descartes (1596-1650) who proposed that animals were no more than 'machines'. Descartes and other 'mechanists' believed that life could be explained fully by chemical and physical principles and properties alone. Nineteenth century adherents of the 'mechanistic' viewpoint included such notable physiologists as Herman von Helmholtz (1821-94), Carl Ludwig (1816-95), Ernst Brucke (1819-92) and Emil Du Bois-Reymond (1818-96).

In 1859 Charles Darwin's (1809-1882) published the 'Origin of Species' with its implication that man could no longer be considered unique: that there was a continuity between man and the animals. Darwinists argued that vitalism should join the phlogiston theory and the Ptolemaic theory of the universe, on the scrap heap of erroneous philosophies[5]. Darwinists maintained that there was no difference between a living and a dead organism, which could not be explained in terms of chemistry.

Claude Bernard (1813-78) did not believe in 'vitalism' but neither did he agree fully with the 'mechanists'. He believed that the hallmark of life was the presence of a 'definite idea' which directed its development. The pioneering clinical chemist, Henry Bence Jones (1813-73) believed that the vital force played a minor role in living processes and that most, if not all, living processes would eventually be understood in terms of chemical and physical laws[6].

Wöhler and the Synthesis of Urea Urea was considered an 'organic' substance, i.e., one that could only be made by a living organism possessing the essential 'vital' force. This metaphorical use of the term 'organic', describes integrated systems having properties that transcend those of their parts, e.g., living entities. It should not be confused with the modern meaning of the term 'organic', i.e. carbon containing compounds. Urea was first isolated in 1799 from urine, by Antoine François, Comte de Fourcroy (1755-1809). The word 'urea' is derived from the French word 'urée ' which was believed to be the 'essential salt' of urine. 'Urée' is derived from the Greek word 'ouron' meaning 'urine'[7].

In 1828, Friedrich Wöhler (1800-82) found that urea, an 'organic' substance, could be synthesized in vitro without any 'vital force' or living organism. Wöhler had discovered that urea could be produced by evaporating an isomeric solution of ammonium cyanate. This was the first 'organic' synthesis, a milestone in clinical chemistry, a bridge between the 'organic' and 'inorganic' worlds, between the living body and the laboratory. This was the first proof that the complex processes occurring within the human body could be understood in terms of chemical procedures that could be carried out in vitro. This work removed the requirement for any mysterious 'vital force' that separated in vivo biochemistry from in vitro chemistry.

Wöhler was born near Frankfurt, Germany in 1800. This was the same year in which Napoleon orchestrated the dissolution of the German Empire; Marie François Xavier Bichat (1771-1802) the French physiologist and surgeon, founding father of the science of histology and major theorist of vitalism [8], published his studies of post-mortem changes occurring in human organs ('Physiological Researches on Life and Death'[9]) and Benjamin Waterhouse became the first U.S. physician to use a smallpox vaccine (on his son).

Wöhler studied medicine, receiving his medical degree in 1823 but his true passion was for chemistry. He gave up medicine and moved to Stockholm to study under Jöns Jacob Berzelius (1779-1848). Berzelius' accurate determination of atomic and molecular weights helped to establish the laws of combination and the atomic theory. He also invented the system of chemical symbols now used universally[10]. Wöhler spent time at Berzelius' laboratory in Stockholm improving his analytical chemistry skills. It was here that Wöhler showed that silver cyanate was a salt of the recently discovered cyanic acid.

Wöhler made the disconcerting discovery that cyanic acid appeared to be identical in composition to fulminic acid which had been discovered by Liebig. Fulminates and cyanates have very different chemical properties and it was assumed that either Liebig or Wöhler had made a mistake. Liebig accused Wöhler of being an incompetent analyst. Unsurprisingly, this unprofessional conduct failed to resolve the paradox. However, in 1826, Wöhler and Liebig agreed to meet in order to examine carefully, their respective analyses. The outcome of this meeting was satisfying for both parties, if somewhat paradoxical: it was concluded that neither chemist had made a mistake in their respective analyses and that, therefore, they must both be correct. It had been shown that apparently different compounds could have the same chemical composition and yet have very different chemical properties. The resolution of this disagreement resulted in the two chemists becoming good friends and to fruitful collaboration in future years. This included a series of experiments that demonstrated how benzaldehyde could be converted into several different compounds, each containing the $C_{14}H_{10}O_2$ group, which they subsequently named the 'benzoyl' group.

These collaborative experiments, together with Berzelius' own work, in which he had failed to detect any difference in chemical composition between racemic and tartaric acids, helped to pave the way for his theory of isomerism, published in 1831. The theory of isomerism postulated that substances could have the same chemical composition and yet have different chemical properties, due to their differing three-dimensional arrangement of atoms[11]. Wöhler had already shown a striking example of isomerism three years earlier, in that urea, extracted from canine urine, had the same chemical composition as did ammonium cyanate.

Following his discovery, Wöhler wrote to Berzelius, '. . .I must tell you that I can prepare urea without requiring kidneys or an animal, either man or dog' [12]. Berzelius replied, 'It is quite an important and nice discovery which Herr Doktor effected and I was indescribably pleased to hear of it'[13]. In his textbook on animal chemistry published in 1831, Berzelius writes, ' Wöhler made a remarkable discovery that urea can be produced artificially'[14].

Despite the fact that Berzelius had recognized the importance of Wöhler's discovery, Wöhler's achievement had little immediate impact. It was a revolutionary discovery that failed to cause a scientific revolution. According to T.S. Kuhn (1922-) the American philosopher and historian of science, revolutions in science occur whenever there is a paradigm change. Kuhn uses the term 'paradigm' to mean a specific set of scientific achievements embodying experimental results and procedures, patterns of theoretical interpretation and methodological orientation[15]. When a paradigm change occurs the accepted theoretical and experimental procedures are questioned and may be discarded or replaced by a new paradigm that fits the experimental observations more closely. For example, Lavoisier's (1743-94) investigations into the nature of combustion caused a paradigm change by displacing the previously widely accepted phlogiston theory.

Some discoveries result in immediate scientific revolution while others do not. Acceptance of Einstein's (1879-1955) special and general theories of relativity was rapid and led to a profound paradigm change. Acceptance of the implications of Wöhler's synthesis of urea was slow by comparison. There was no revolution, no sudden paradigm shift. It is unclear exactly how or why scientific revolutions occur. Do they occur internally, because of the accretion of inconsistencies in the currently accepted paradigm, or are they caused by external forces, e.g. social and political upheavals, which provide the impetus for the reinterpretation of these same anomalies? Does communications technology, e.g. the Internet, which increases the flow of information also increase the likelihood of paradigm change?

Berzelius rationalized Wöhler's discovery by suggesting that urea was on the borderline between the organic and the inorganic, i.e., that it could be produced both artificially and naturally. He modified, rather than discarded, the existing vitalist paradigm because he would not accept fully the implications of Wöhler's work.

The German physiologist, J. Müller, took up a similar position. After, systematically discounting the numerous claims to organic synthesis made by others, Müller accepted Wöhler's work as valid. However, he cast Wöhler's discovery into a vitalistic world-view, by redefining the very nature of urea: 'However, urea is placed at the extreme border of organic substances and is more of an excretion than a component of the animal body. Perhaps urea is not at all a compound with characteristic properties of organic products'[16].

Despite the wishful thinking of historians of clinical chemistry[17], 'vitalism' was not abandoned following Wöhler's synthesis but continued into the next century. Others explained away Wöhler's discovery as little more than isomerism: a rearrangement of atoms rather than as an organic synthesis per se. Stereochemical specificity, the ability to distinguish between alternate enantiomers was considered one of most striking features of biological chemistry[18]. Indeed, McKie[19] has argued that Wöhler's preparation of urea from ammonium cyanate was a 'transformation', rather than a synthesis per se. According to McKie, a synthesis is 'the compounding of a substance from the elements that compose it' and points out that the cyanate, as it was prepared in Wöhler's day, originated from organic matter. McKie considers the first true organic synthesis to be that of acetic acid by Kolbe (1818-84) in 1845[20]. However, Mikuláš Teich[21] dismisses McKie's thesis, restoring Wöhler to his rightful position as the first chemist to synthesis an organic substance.

It was not until P. E. Berthelot (1827-1907) published his studies on chemical synthesis in 1869 that the importance of Wöhler's work was realized fully. Wöhler's discovery was revolutionary. It implied that Berzelius was incorrect when he asserted that 'In living nature the elements seem to obey entirely different laws than they do in the dead. . .'[22]. This statement is taken from his textbook that was first published in 1827, the year before Wöhler's synthesis of urea. This viewpoint was repeated in subsequent editions, including the last one published in 1847. This implies that Berzelius, one of the age's greatest chemists, held his vitalistic views well after Wöhler's clear demonstration that they were incorrect. Berzelius seemed more interested in the contribution of Wöhler's work to his own emerging theory of isomerism than to its implications for the doctrine of vitalism. Berzelius postulated that an entirely new force was responsible, the 'catalytic force', which was common to both organic and inorganic matter.

Some adherents of vitalism attempted to minimize the significance of Wöhler's discovery. For example, Johannes Müller (1801-58) argued that urea was not really an animal product after all, but was instead a product of excretion. Charles Gerhardt (1816-56) took a similar stance, arguing that " . . . only the vital force operates to synthesize". He maintained that urea was a decomposition product formed by purely chemical (non-vitalistic) forces and that this 'decomposition' was a type of in vivo combustion.

Liebig was more pragmatic in his approach. His collaborative studies with Wöhler on benzoyl derivatives had helped to establish the theory of radicals. Whenever possible, Liebig would explain chemical reactions occurring in agricultural chemistry or in animal chemistry, in terms of the behaviour of molecules, without resort to any 'vital' forces. However, when he could not explain a result, he was not averse to resurrecting the 'vital force' to explain what had occurred. During his lifetime (1803-73) the majority of metabolic pathways were unknown. In general, only the initial and final products were known but not the intermediate metabolites, knowledge of which was crucial to understanding experimental observations in terms of a series of incremental molecular changes.

In 1853, Claude Bernard discovered that glycogen was formed by the liver[23]. This contradicted yet another tenet of vitalism, i.e. that only plants could synthesize complex compounds which were subsequently consumed by animals. In 1860 Berthelot (1827-1907) published a book that presented numerous examples of the synthesis of organic compounds from the elements[24]. Hans Driesch (1867-41) was perhaps the last of the 'vitalists', insisting that the functions of protoplasm could not be fully explained mechanistically.

Annual reports or reviews of a particular area of science are a commonplace and a widely accepted method of synthesizing and putting into perspective recent advances. The original idea of writing annual reviews was that of Thomas Thompson (1773-1852) who published an annual retrospective of European chemistry in each January issue of Annals of Philosophy. Berzelius produced a similar set of retrospective reports for the Stockholm Academy from 1822-48. It was Wöhler who translated these reports into German, making them far more widely accessible[25].

A vital chemist Wöhler returned to Berlin in 1825 to teach chemistry at a technical school. It was here that he first synthesized urea in 1828. He also studied uric acid and cocaine, invented a method for purifying nickel and worked with Justus, Baron von Liebig (1803-1873) on benzaldehyde which contributed to Liebig's development of the theory of radicals.

Wöhler was appointed professor of chemistry at the University of Göttingen in 1836 where he remained for the rest of his life. He was a very unusual professor: he was not only an outstanding teacher, but was also very interested in his students' welfare. In his later years Wöhler trained over twenty American students who came to his laboratory in Göttingen for advanced training in chemistry. He wrote several chemistry textbooks and edited Liebig's 'Annals of Chemistry', the most important chemistry journal of that time.

In 1839 Wöhler published an anonymous spoof article in collaboration with Liebig in the journal Annalen that mocked Louis Pasteur's (1822-95) assertion that alcoholic fermentation was caused by living yeast cells[26]. In this satirical paper, Liebig and Wöhler described their observation, under the microscope, of many small animals shaped like tiny distillation vessels. These animals were observed consuming sugar and digesting it into carbonic acid and alcohol, which were then excreted separately. They maintained that the entire process was clearly visible under the microscope!

Wöhler died in 1882, the same year as did Charles Darwin (1809-1882). According to Fruton and Simmonds[27], 'The ultimate goal of biochemistry is to describe the phenomena that distinguish the 'living' from the 'non-living' in the language of chemistry and physics'. Wöhler's synthesis of urea began the quest for this goal by removing any requirement for mysterious, unexplainable 'vital' forces. At the beginning of the nineteenth century, organic chemistry was, in Wöhler's words, " . . . like a dark forest with few or no pathways" . Wöhler began the task of opening up the forest by taking the first step on the pathway to understanding the chemistry of life.

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