Clinical Chemistry 49:10
1696–1707 (2003)

History

Justus Liebig and *Animal Chemistry*

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Justus Liebig was one of the individuals making chemistry almost a German monopoly in the 19th century. At Giessen he established the first organic chemistry laboratory and offered a systematic course for training new chemists. His comprehensive survey of plant nutrition changed the nature of scientific agriculture. In a study of animal chemistry, Liebig treated physiologic processes as chemical reactions and inferred the transformations from the chemical properties of the elements and compounds in laboratory reactions. He constructed hypothetical chemical equations derived from the formulae of the participating compounds. Liebig generalized that all organic nitrogenous constituents of the body are derived from plant protein and demonstrated how the application of quantitative methods of organic chemistry can be applied to the investigation of the animal organism. Liebig's theories were attractive, but his method of converting one substance to another by moving atoms around on paper was speculative because of the lack of knowledge as to how the elements were arranged. His dynamic personality helped win widespread acceptance by many, but others were antagonized by his wishful thinking and speculative excesses. Liebig's views on catalysis and fermentation brought him into a controversy with Louis Pasteur. Liebig's *Animal Chemistry* stimulated an interest in clinical chemistry because it introduced a quantitative method into physiological chemistry. However, the isolated pieces of test results on blood and urine were unconnected and did not fit anywhere. Physicians found that chemistry was not helpful at the bedside and they lost interest in its application.

Both chemists and physiologists accepted vitalism as a way of explaining vital phenomena that could not be explained in physicochemical terms. But although chemists believed that vital force would steadily erode as chemistry advanced, many physiologists denied that chemistry even had a useful role in the study of life functions. Chemists and physiologists have long held opposing views about the nature of the vital functions and whether they could be explained in strictly physicochemical terms. Inroads by chemistry and its methods into medicine and the pathology of disease were strongly and widely resisted. Genuine progress depended on a more detailed knowledge of the chemical nature and composition of animal matter and functions *(1)*.

At the end of the 18th century, organic chemistry was mostly a descriptive science engaged in isolating, identifying, and with newly developed methods, techniques, and improved facilities, in analyzing a great number of compounds from plant and animal sources. The "animal chemistry" of the late 18th and early 19th centuries had sought to isolate organic substances from plant and animal sources, in a state unchanged by the process of isolation. Procedures had to avoid destructive calcination (distillation until dry), a common procedure in organic chemistry for centuries, because it yielded much the same complex mixtures of poorly characterized degradation products and weighed as fractions of gas, oil, phlegma, and residue from everything studied. It was believed that chemical identification of these materials and their properties would lead to an understanding of biological organization and physiological function *(2)* and reduce the empirical element in medicine.

It seemed that the conversion of vegetable foods into animal substances could be investigated by comparative chemical examination of animal and vegetable substances. In 1789, Antoine François Fourcroy (1755–1809) discovered a nitrogenous material in cruciferous plants with the same properties as the albumin of egg white. Other investigators obtained nitrogenous extracts from numerous plants and vegetables whose appearance, solubility, and color reactions resembled products of animal origin. The terms albumin, fibrin, and casein were soon applied to these nitrogenous plant substances. The presence of nitrogen in many vegetables could account for its presence in herbivores without having to assume that the animal absorbed it from the atmosphere. Fourcroy concluded that animal substances were more complex than vegetables, but he could not distinguish between them with chemical tests *(3)*.

Food shortages and high prices caused by the wars

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Received April 11, 2003; accepted June 27, 2003.

between France and England and the political upheavals that carried over into the 19th century led to the study of the chemical constituents of plant and animal food. These foods were extracted with water, acids, alkalies, alcohol, and ether, and the extracts were subjected to various procedures in an attempt to isolate pure substances. Many of these techniques, thought to be universally applicable, had adverse effects on protein material. They coagulated irreversibly at moderately increased temperatures and were damaged by acid or alkali.

By the early 19th century, solvent methods for separation and characterization of the "animal" substances became so highly developed that they emerged as fundamental tools for determining the composition of the fluids and solids of animals and plants *(4)*. As methods evolved for the separation of proteins by the precipitating action of various reagents, the diverse character of proteins from different sources began to be recognized.

During the first half of the 19th century, the Industrial Revolution was drawing increasing numbers of laborers to jobs in the cities. With fewer agricultural workers to feed them, there was a need for increased food production and for an understanding of the chemical changes during digestion and assimilation in living systems *(5)*.

The first serious attempt at the quantitative analysis of organic compounds was made by Antoine Laurent Lavoisier (1743–1794). Whereas Lavoisier had burned a few inflammable substances, using mercuric oxide as the source of oxygen, Joseph Louis Gay-Lussac (1778–1850) and Louis Jacques Thenard (1777–1857) revolutionized organic analysis in 1810 by adopting an oxidizing agent, potassium chlorate. Accuracy with this dangerous procedure was very much dependent on the operator's skill. In 1815, Gay-Lussac proposed the use of the less dangerous and more reliable copper oxide. The cumbersome apparatus was transformed into a safer and simpler horizontal arrangement by the Swedish physician-chemist, Jöns Jakob Berzelius (1779–1848). Uncertain volumetric estimations were replaced by the direct weighing of carbon dioxide and water collected by absorption and condensation.

The final major modification in combustion analysis was made in 1830 by Justus Liebig (1803–1873), who is the subject of this report. The simplifications he introduced eliminated much of the tedium and extraordinary skill formerly required. Not only could many more compounds be analyzed in far shorter time than before, but he could entrust analyses to his students. Because he had many students, his laboratory began turning out hundreds of analyses annually. Liebig's method and combustion apparatus were so well conceived that reliable analyses became commonplace for the determinations of carbon and hydrogen in fats, sugars, and other relatively small molecules, and by 1840, a relatively large number of organic compounds had been analyzed and their elementary composition determined. Heating by spirit lamp or charcoal gave way to coal, then to gas with the Bunsen burner in the 1860s, and to electrical heating in the 20th century. Liebig's method for determination of carbon and hydrogen in elementary analysis remains in use today.

Mulder's Protein Radical

The new awareness of the importance of the albuminoid substances made it inevitable that the improved analytical methods noted above would be used for their analysis. There was little agreement among chemists as to the formulation of organic compounds, and no unifying principle by which molecular magnitudes could be established. A great step forward was made by Liebig and Friedrich Wöhler (1800–1882) in 1832 when they introduced the concept of the chemical radical. In their investigation of the oil of bitter almonds (benzaldehyde), they demonstrated that a whole series of related compounds could be formulated in terms of one uniform group of atoms, which they called the radical, e.g., benzaldehyde, benzoic acid, and benzoyl chloride. This observation gave rise to an enthusiastic search for other series of compounds that could be formulated in a similarly simple fashion and led to the clear conception of organic radicals. Another radical was postulated by Liebig and Wöhler when in 1838 they assumed that urea preexisted in uric acid. By subtracting the formula of urea from that for uric acid, they derived a hypothetical body, *uril.* Accordingly, uric acid was uril+urea.

The Dutch physician-chemist, Gerrit Jan Mulder (1802–1880), whose interests included nutrition and agricultural chemistry, attempted to fashion this new idea to the study of albuminous substances to gain a more definitive characterization. By then, protein was already regarded as a chemical molecule of great size. His analyses of fibrin, serum albumin, and casein, as well as egg albumin and silk, indicated that—although they differed in their chemical and physical properties—they all had approximately the same ultimate composition, with identical proportions of carbon, hydrogen, oxygen, and nitrogen, and differed only in the small amounts of sulfur and phosphorus with which they were combined. On the basis of his elementary analysis he concluded that the albuminous substances are compounds of differing quantities of sulfur and phosphorus with an organic radical nucleus or root substance, which he called "protein" (Greek: *proteios,* of the first rank or position) *(6–8)*. The word had been proposed by Berzelius *(9)*, for many years the international authority for new chemical terms.

Justus Liebig

Justus Liebig (Fig. 1) *(10–12)* was one of the forces making chemistry, in which France had led the way in the 18th century, almost a German monopoly in the 19th century. However, science recognizes no national boundaries. Pneumatic chemistry was introduced by English and Scottish chemists in the late 18th century, and phlogiston, the German name for the principle of inflammability

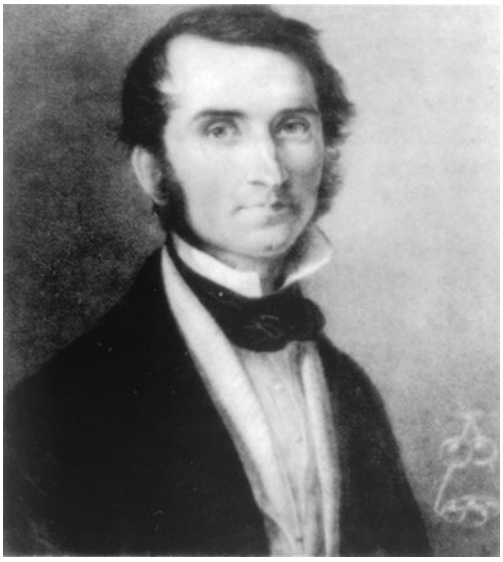

Fig. 1. Justus Liebig at age 36, at the height of his powers. Courtesy of The Liebig-Museum in Giessen.

invented by Georg Ernst Stahl (1660–1734), dominated chemistry before Lavoisier.

Liebig had received his doctorate from the University of Erlangen in 1822 under Karl W.G. Kastner (1783–1857). Although Kastner was one of the most eminent chemists of the period and author of several widely used books, Liebig considered his teacher unskilled in analyses and unable to provide comprehensive chemical instruction. Liebig decided he would have to go elsewhere to complete his education, yet it was on the recommendation of Kastner that he obtained a grant from Grand Duke Louis I of Hesse-Darmstadt to study in Paris. There he attended the lectures of Gay-Lussac and Thenard and learned how to analyze organic materials. He encountered a rigorous, quantitative experimental chemistry and learned for the first time some of the general principles connecting his knowledge of particular compounds and processes. It was unlike anything he had found in Germany, where *Naturphilosophie,* an essentially speculative philosophy, had turned away from quantitative chemical methods in biology and medicine.

While in Paris, Liebig presented a paper on silver and mercury fulminates to the Académie des Sciences in December 1823. He had investigated fulminate of mercury while at Erlangen. Liebig's report attracted the attention of Alexander von Humboldt (1769–1859), the renowned natural scientist, intellectual, and world traveler, and he arranged for Liebig to work with Gay-Lussac, a close friend. Liebig mastered methods of analysis and learned to pursue investigations systematically. Collaboration with Gay-Lussac led to a memoir on the fulminates. Humboldt was impressed with Liebig's dangerous work on the analysis of silver fulminate and recommended to the Grand Duke that he provide Liebig with an academic position.

At about the same time, Wöhler reported an analysis of silver cyanate with the same composition that Liebig had found for silver fulminate. When each was able to confirm the other's analysis, it led to the realization that two chemical compounds with entirely different properties could have the same elementary compositions, differing only in the manner in which the elements were arranged. This relationship was an example of isomerism (Greek: *isomeros,* composed of equal parts), a term proposed by Berzelius in 1831.

Returning to Germany in 1824, Liebig, only 21 years of age, was appointed extraordinary professor in the philosophical faculty at the University of Giessen by the Grand Duke, bypassing the election process of the faculty. Only 1 year later, Liebig succeeded to the chair for chemistry. Liebig was determined to make the learning opportunities in Gay-Lussac's laboratory available to a larger number of students. Previously, practical laboratory exercises were neglected in the universities, and students were taught only theory. Experiments were almost always limited to demonstrations by the instructors and their assistants.

The Laboratory at Giessen

Liebig's proposal for a pharmaceutical institute was turned down, but he was permitted to set one up on his own. By 1827, chemistry dominated the instruction. This private pharmaceutical institute was separate from his state-supported teaching at the University, although there must have been some overlap of activities. By 1831, Liebig had established a national and international reputation. In 1833, his private school was combined with his official university course. During the following years the number of students in chemistry exceeded those in pharmacy. With his growing fame and invitations from other universities to teach there, his complaints about salary and inadequate appropriations for laboratory expenses were periodically satisfied by the government.

Liebig's Pharmaceutical Institute was a remodeled guardhouse of a former military barracks *(12)*. The lower floor housed the laboratory and the service rooms for the balance, chemical supplies, glassware washing, and Liebig's laboratory assistant. The apartment in the upper floor was occupied by Liebig and, later, by his family.

The laboratory was an unventilated room with a large charcoal stove in the center. There was no chemical hood. If necessary, the windows and outside door were opened for ventilation. Chemists at that time had to produce all of the necessary reagents themselves or isolate them from commercially available impure raw products. Water for chemical experiments and for rinsing glassware was provided primarily by rain water, which was passed through a sand and gravel filter and collected in cisterns. Because of the institute's limited budget, Liebig bought most of the equipment and chemical supplies and paid his assistant from his own modest salary. A larger analytical laboratory (Fig. 2) with tables along the walls, cupboards and drawers below, shelves above, and scientists at work back

Fig. 2. Liebig's analytical laboratory in Giessen.
From a sketch by Trautschold and v. Ritgen, 1840. Courtesy of the Library of the Justus Liebig University, Giessen.

to back was built for him in 1839, after he received and turned down an offer from the University of St. Petersburg. In 1852, he left for the University of Munich, which offered better facilities and freed him from teaching responsibilities. The Giessen laboratory continued to attract students. The building was restored in 1920 and opened as a museum dedicated to the memory of Liebig. Damaged during World War II, it was reopened in 1952 and is today among the world's most important chemistry museums.

The Course of Instruction

Liebig established the first systematic laboratory course designed expressly to train new pharmacists. This laboratory was the beginning of a whole new mode of training scientists. Through a carefully planned program of exercises, students could progress systematically from elementary operations to independent research under the guidance of an established scientist. Liebig made organic analysis teachable and chemical research learnable. In his first years in Giessen, the emphasis of the work in the laboratory was in developing and perfecting both teaching methods and elementary analysis. Liebig's analytical methods were based on those pioneered by Berzelius and Gay-Lussac, modified for use by young chemists and others with only moderate analytical skill *(1, 10)*. His program of research and instruction was the beginning of a whole new mode of training scientists, and it became the model for others in Germany and elsewhere. This is where the development of the teaching, research, and technology of modern chemistry began.

Before this time, science had been done in laboratories of general chemistry, physiology, pathology, and clinical medicine. The decisive change came when Liebig set up his teaching laboratory at Giessen. Other chemists, after studying in Paris, also set up centers of chemical training, and by 1840, owing to the growing interest in applications of chemistry in agriculture and industry, a steady flow of well-trained chemists was emerging in Germany. Whereas chemistry in the previous century emphasized its relation to pharmacy, metallurgy, and mining, it now became a scholarly subject taught in universities to future highly specialized professionals. At the core of these programs was laboratory experimentation. This was in sharp contrast to the teaching of chemistry in the 18th century in the universities where chemistry had been part of the medical curriculum *(13)* to train chemists (apothecaries) to prepare the medications prescribed by the physician. The crucial separation of chemistry from medicine—in spirit and doctrine—occurred when chemistry

moved from the medical faculty to the philosophical faculty as an independent discipline *(14)*. Chemistry had been a didactic subject taught mainly by physicians in the medical schools of Europe until Liebig changed it into an experimental laboratory discipline in its own right. Liebig's laboratory attracted students from all over, and Giessen became the world's foremost institution for chemical instruction.

While at Giessen, Liebig was asked by Phillip Lorenz Geiger (1785–1836), a Heidelberg pharmacist, to join him as co-editor of *Magazin für Pharmacie* (renamed *Annalen der Pharmacie* in 1832). Geiger needed Liebig to verify the accuracy of chemical statements in articles submitted for publication. After Geiger's death, Liebig changed the name of the journal to *Annalen der Chemie und Pharmacie* (1840). As editor he helped set standards that made it the leading journal of chemistry of his time. After Liebig's death in 1873, the journal was briefly known as *Justus Liebig's Annalen der Chemie und Pharmacie,* then as *Justus Liebig's Annalen der Chemie.* In 1979 it became *Liebig's Annalen der Chemie,* and in 1995 *Liebig's Annalen,* which ceased publication in December 1997 and was absorbed into the *European Journal of Organic Chemistry.*

Liebig did as much as anyone to bring about the era of large-scale research, in which the ability to organize became as critical as the ability to conceive and carry out experiments. Between 1830 and 1840, he was at the very center of the rapidly growing field of chemistry. He was one of the chief pioneers in physiological chemistry and had a long-lasting influence on the younger generation of scientists and physicians, many of whom studied at Giessen and then went out to develop the new discipline at various hospital centers in central Europe. Liebig helped establish the independent and scientific status of the chemist.

Animal Chemistry

Justus Liebig appreciated the need to link chemistry, especially organic chemistry, to physiology, and he worked hard to put organic chemistry to the service of agriculture and physiology. His book *Organic Chemistry in Its Application to Agriculture and Physiology* (1840) was one of the most important books in scientific agriculture. An expanded seventh edition appeared in 1862. He gathered the scattered conclusions of previous investigators into the most comprehensive survey of the problems of plant nutrition ever presented, using his own findings to decide between alternative theories. Liebig refuted the widely held theory that humus, the product of the decay of plant matter, formed the main nutrient substance for plant growth and supported the view that the source of the carbon assimilated into plant substances is the atmosphere. The most important plant function, he stated, was to separate the carbon and oxygen of carbonic acid in the atmosphere, releasing the oxygen and assimilating the carbon into compounds such as sugar, starch, and gum *(10)*.

Although Liebig's general picture of plant physiology was not original, one of his main purposes was to persuade botanists and physiologists that they must pay more attention to chemistry if they were to make further progress. He stressed that knowledge of the composition of soils was the basis for the whole system of rational agriculture. Although the first edition contained mistakes, inconsistencies, and disorder, its appearance completely changed the nature of scientific agriculture. Before 1840 it was generally believed that both plant and animal life were dependent on the circulation of an organic, previously living material. It was now agreed that the nutrient substances of plants were inorganic *(10)*. The first serious attempt to apply chemistry to agriculture had been the series of lectures by Humphry Davy (1778–1829) in 1813 at the Royal Institution in London. *Elements of Agricultural Chemistry in a Course of Lectures for the Board of Agriculture* remained the standard work until Liebig's publications *(15)*.

Liebig next began a study of animal chemistry, confident that his knowledge of the chemical properties of organic compounds would enable him to infer the transformations occurring within living systems. Liebig reasoned that he could predict the behavior of the substances within living organisms from knowledge of the chemical properties of elements and compounds in laboratory reactions.

Liebig and his students verified and extended Mulder's results. Liebig isolated a nitrogenous plant substance exactly identical to casein in composition of organic elements, proportion by weight, and properties. In a letter to Wöhler, he made the analogy of plant albumin, fibrin, and casein, all identical, and identical with the animal proteins that have the same names *(8)*. Thus, the animal obtained its protein ultimately from a diet of vegetable substances, either directly or indirectly through the bodies of other animals *(16)*.

Liebig elaborated on his physiological ideas in *Die Organische Chemie in Ihrer Anwendung auf Physiologie und Pathologie* (*Animal Chemistry, or Organic Chemistry in Its Application to Physiology and Pathology*) (1842). The book was an extraordinary success. Translations into English, French, and Dutch appeared quickly. The "Introduction" to the English translation, written by Frederic L. Holmes in 1964, is the best analysis and revue of the book, the reception it received, and the personalities involved.

Liebig described fibrin and albumin as the chief ingredients of blood *(17)*. "Chemical analysis has led to the remarkable result, that fibrine and albumen contain the same organic elements united in the same proportion, so that two analyses, the one of fibrine and the other of albumen, do not differ more than two analyses of fibrine or two of albumen respectively do, in the composition of 100 parts." Although differing in external properties, in chemical composition they are identical.

Liebig generalized *(18)* that vegetables produce protein, from which all the diverse constituents of the various

tissues and parts of the animal body, e.g., feathers, claws, globules of the blood, fibrin, membrane and cellular tissue, arteries, and veins, are produced by the vital force by the addition or subtraction of the elements of water or of oxygen of the atmosphere.

"The true starting-point for all the tissues is, consequently, albumen; all nitrogenized articles of food, whether derived from the animal or from the vegetable kingdom, are converted into albumen before they can take part in the process of nutrition."

Liebig tried to figure out the actual chemical transformations that the three classes of nutrients undergo within the animal body and developed a new comprehensive theory of chemical processes in living organisms. To depict these more concretely, he constructed hypothetical chemical equations derived from the formulae of the participating compounds and treated physiological processes as chemical reactions subject to the laws of chemistry and physics, as he and his colleagues had been doing to interpret the reactions of organic compounds in their laboratories. Liebig thought that he could demonstrate how quantitative organic chemistry could be applied to problems of physiology that physiologists had failed to solve *(10)*.

Where Mulder had suggested a single basic "protein" radical from which all of the plant and animal substances are formed by slight modifications, Liebig postulated compounds preformed in plants that were equivalent to each of the principal nitrogenous constituents of the blood and tissues of animals. Before being assimilated almost intact, he believed that these proteins required only minor alterations in form, not changes in their composition *(19–21)*. Sugar, fat, and albumin would always originate in plants because animals might modify, but were believed incapable of synthesizing, compounds of this complexity.

In France, Jean-Baptiste André Dumas (1800–1884), the outstanding French chemist of his time, went even further in 1844 when he asserted that animals cannot synthesize any substances, but " . . . receives and assimilates almost intact the neutral nitrogenous substances which it finds fully formed in the animals and plants that form its food" *(8)*. It can only decompose them through successive oxidations, which release heat and mechanical work. It seemed to chemists like Liebig and Dumas that to search for chemical transformations during the process of digestion and assimilation was to unnecessarily complicate the great simplifying generalization that the substances involved were all alike *(19, 21)*.

Liebig's Concept of Stone Formation

Henry Bence Jones (1813–1873) had come to Liebig's laboratory in Giessen for 6 months soon after passing the examination for Licentiate of the College of Physicians in 1841 and became a close friend of his teacher. Jones was greatly impressed with Liebig and his views on animal physiology and based most of his own work on Liebig's concept of the oxidative metamorphosis of tissues, i.e., the interconversion of biological molecules.

In his first book, *On Gravel, Calculus, and Gout: Chiefly an Application of Professor Liebig's Physiology to the Prevention and Cure of These Diseases* (1842), Jones used Liebig's concept to explain the causes, treatment, and prevention of formation of bladder stones, a common ailment at that time. Urea was defined as arising from uric acid. Bence Jones's approach to preventing the formation of uric acid stones was to increase the rate of oxidation of uric acid to the more soluble urea by increasing the supply of oxygen. Consequently, he emphasized vigorous exercise and limiting the intake of nonnitrogenous foods, which were thought to inhibit such oxidation, and administered alkaline medication to keep the uric acid in solution, where it was more readily in contact with oxygen.

Because of the strong impression made by Liebig's work, Bence Jones always preferred the findings of German chemists over those of the French. This led him to ignore or even to reject some important contemporary findings in physiological chemistry. For example, he was not satisfied with Claude Bernard's (1813–1878) experiments demonstrating that pancreatic juice saponified neutral fat, and he cited the contrary opinion of a German researcher *(22)*. The weak point in his and Liebig's work was a too-direct application of the laws of chemistry to the complex phenomena of the human body.

According to Liebig's chemistry of physiological processes, substances in the tissues and in food were broken up and their atoms then rearranged into new combinations—by the vital force. Because, in the majority of biochemical reactions, only the initial and final products were known, vital force was an easy explanation for the intermediate steps connected with secretion and animal metabolism.

Liebig's approach to converting one substance to another by moving atoms around on paper was convenient and attractive because it was based on a balanced system of oxidative reactions, all fitting neatly together on a molecular "balance sheet" to explain animal metabolism. The addition and subtraction of atoms looked plausible on paper, but because of limited knowledge of chemical structure, were obviously speculative.

Response to *Animal Chemistry*

Liebig's *Animal Chemistry* produced sharply divergent reactions. Probably the most constructive criticism came from the Hannover physician, Otto Kohlrausch (1811–1854). Although he rejected Liebig's fundamental claims, he conceded that his work had revealed the interdependence of an animal's metabolism, respiratory exchanges, intake of food, and production of heat *(1)*. "Liebig shows us a path which if properly followed can lead to the most fundamental method of observation in the entire field of medicine" *(23)*.

Liebig's dynamic and commanding personality and his vigorous and vivid literary style won widespread accep-

tance for his ideas. Others were antagonized by his wishful thinking and speculative excesses, which went far beyond the available experimental evidence. Although frequently in error, the fault was not in his observations but in his unrestrained enthusiasm *(16)*. It also brought criticism and bitter conflicts with others. Engaging in every controversy and scientific dispute over rival chemical theories and rarely able to preserve a distinction between intellectual disagreements and personal attacks, Liebig became embroiled in furious literary polemics. He often used his publications to rush into print to forestall another organic chemist pursuing the same course, to publicize his own views, and to denounce and discredit other chemists, German and foreign, with whom he disagreed—a practice that earned him many enemies. Quick to charge plagiarism and claim rights of priority, he was not inclined to credit the work of others that contributed to his own conclusions. He rarely underrated the importance and novelty of his own contributions. Liebig's disputes with Dumas were part of the rivalry between German and French chemists in the 1840s to dominate organic chemistry. To regard as their own ideas that were largely either speculative or generally known was a weakness of both groups *(24)*.

Liebig treated his scientific differences with Dumas as political struggles. There was a strong emotional overlay driving Liebig in this rivalry. He and Dumas had opposed each other repeatedly on both theoretical and experimental questions, and each had resorted to personal criticisms in his arguments. Liebig's heated reproaches drove the disputes to a depth out of proportion to their intellectual differences. Dumas soon became the formidable opponent of the school of chemistry headed by Berzelius and Liebig, for whom he was more than a match. Berzelius wrote in 1831 to Liebig that Dumas "does all to shine and it seems little to him to learn the truth." Liebig had to admit that "it always annoys me that this fellow, in spite of his unclean, impossible and bad way of working, yet with the devil's help (demongeachtet) fetches masterpieces out of his sleeve" *(25)*.

Berzelius Reviews Liebig

In his critique of *Animal Chemistry* in 1843, Berzelius accused Liebig of using his considerable powers of persuasion to make plausible hypotheses appear as proven facts. "This easy kind of physiological chemistry is created at the writing desk, and is the more dangerous, the more genius goes into its execution, because most readers will not be able to distinguish what is true from mere possibilities and probabilities, and will be misled into accepting as truths probabilities that will require great effort to eradicate after they have become imbedded in physiological chemistry. To the extent that it is easy in this manner to do physiological chemistry, which has many chemical facts with great possibility of combination, it is likely that the aspiration to be the first to bring this *probability-physiology* to market will produce conflict over priority, to the detriment of science" *(8, 26)*.

These comments were all the more devastating because Liebig had dedicated the book to Berzelius. He hoped to associate Berzelius's prestige with his own work and was quite anxious for a good review. Liebig was the most likely candidate to succeed Berzelius as the leading spokesman of organic chemistry and probably expected that Berzelius's blessing would help him achieve that status. Liebig had called himself Berzelius's "adoptive son", and Berzelius his "fatherly friend." However, Liebig was impatient for his inheritance. He remarked in 1839 to Wöhler: "It is saddening to see how a bright flame slowly expires. Why doesn't . . . [Berzelius] retire and leave the arena to those who still have something to win?" Berzelius's refusal to pass the torch to him stung him more deeply than criticism from anyone else, and he responded with appropriate bitterness *(27)*.

Reversal of Mulder

The protein theory of Mulder suffered a setback when Liebig and his students were unable to isolate the hypothetical protein radical free of sulfur and phosphorus, even after repeating Mulder's own methods. In 1845, Liebig wrote to Wöhler: "After so much has been prattled and written about protein and protein oxide, it is a source of despair to have to see that there is no such thing as protein" *(8)*. In 1847, Liebig dismissed the protein theory as being supported by erroneous observations and misinterpreted significance and blamed Mulder completely for the false direction followed by animal chemistry for the previous 10 years. In addition, he charged that Mulder's theory of the identity of the albuminous animal and plant compounds had led to the false belief that nutrients are assimilated without chemical change. Whereas in 1841 Liebig had praised Mulder's work as "the most remarkable, the most interesting, and the most useful in chemistry," in 1847 he called him incompetent for not seeing that his once-praised methods were no longer adequate *(28)*. Liebig's repudiation of Mulder's "protein hypothesis," and Mulder's defense of it, was one of the most distressing incidents in either of their lives and revealed an aggressive and uncompromising side to Liebig's character *(16)*.

Eventually, Dumas's more exact method of analysis of nitrogen in organic compounds demonstrated decided differences in the elementary composition of many of the then known proteins, e.g., a higher nitrogen content in fibrin than in egg albumin *(8)*. This helped dispose of the notion of their identity and the idea that an animal could obtain all of its many distinctive and definitive constituent proteins by direct assimilation from its plant food.

A Beginning for Clinical Chemistry

The discoveries of new substances in the blood and urine in health and disease that accompanied the beginning of scientific medical research and the development of or-

ganic and physiological chemistry spawned a wave of interest in clinical chemistry as a new recognizable identity in the late 1830s and 1840s. There followed a systematic search for pathological changes in the chemical composition of body fluids to guide medical diagnosis, follow the course of the disease, and control therapy. A search for chemical explanations of biological phenomena became a major preoccupation of leading scientists. The change was brought about mainly by Liebig's *Animal Chemistry.* The book was significant for the development of clinical chemistry because it introduced a quantitative method of observation into physiological chemistry and thereby encouraged doctors to apply quantitative analysis to the diagnosis of diseases *(29, 30)*.

In the 1840s, most of the new professorships in organic chemistry in Germany were held by former students of Liebig, who was influential in their appointment. A key location joining in the almost simultaneous beginning of clinical chemistry in the German-speaking countries was Würzburg, where Johann Joseph Scherer (1814–1869) was director of the first independent hospital laboratory in the Juliusspital (Julius Hospital) of the University of Würzburg. The special feature of his university appointment in 1842 was the close relationship with the university hospital and the requirement that he teach organic chemistry in connection with chemical investigations of blood and urine from the patients admitted to the hospital. His professorship, the first such by the Medical Faculty, became a university chair in 1847 and was the first academic position devoted entirely to this new discipline. Scherer was the first to use the term "clinical chemical laboratory" (klinisch-chemischen Laboratorium) in the foreword of his monograph *Chemische und Mikroskopische Untersuchungen zur Pathologie* (1843).

"Pathological chemistry" was the usual term in German-speaking areas at the time. Scherer, a physician, had studied chemistry and then spent 1 year in Liebig's laboratory in Giessen, with the emphasis on quantitative methods in the application of chemical knowledge to problems in medicine *(31, 32)*.

Another of Liebig's students was Max Josef Pettenkofer (1818–1901), who was educated as a pharmacist and studied medicine in Munich. In 1844, working in Scherer's laboratory in Würzburg, Pettenkofer developed his color test for bile acid in urine and began studies that led to his discovery of a new nitrogenous substance in human urine later that year in Liebig's laboratory. It was precipitated by zinc chloride as a crystalline double salt *(33)*. Some time later, Liebig named it "kreatinin."

In 1847, Pettenkofer was appointed extraordinary professor of physiological and pathological chemistry at the University of Munich. He tried to convince the clinicians of the practical importance of chemistry in clinical medicine, but was disappointed by their lack of interest and reluctance to accept help from chemistry unless it could be used as a "luxurious embellishment for a clinical lecture." In a letter to Liebig in 1849 he complained: "The reagent box now holds the same position in the clinics as the crocodile and basilisc used to in the stalls of those itinerant Aesculapians. We must have it, but we can get no use out of it" *(23, 34)*.

New chemical tests provided a great deal of data for many constituents in urine, and volumetric (titrimetric) methods replaced the laborious gravimetric techniques. Two such analytes were chloride and urea, capable of simultaneous analysis with Liebig's procedure. Liebig's method for this dual analysis (1853) was the first "quantitative" titrimetric chemical method that could be used easily at the bedside. Qualitative tests for urine had been in use for many years but were helpful only for pathological concentrations of albumin and sugar. As for normally occurring substances in urine, only a change of concentration or in daily output might be of diagnostic value *(23)*.

Liebig was the first to use mercuric nitrate solution for the determination of chloride *(23, 35)*. He noted in 1853 that mercuric ions produce a white precipitate with urea in neutral solution, but that this precipitate does not form in the presence of chloride ions. Consequently, with urea as indicator, he titrated chloride with mercuric nitrate solution until the chloride was bound, but not precipitated, as mercuric chloride, and the mixture showed an opalescence or precipitate attributable to the interaction of mercury and urea at endpoint. In this method, continuing the titration and while urea remained in excess, a drop of the mixture added to sodium carbonate solution in a test tube would produce a white precipitate of the mercury–urea complex. At endpoint all of the urea would be complexed. An excess of mercury would be present, and a yellow-brown coloration or precipitate of mercuric oxide would be formed when a drop was tested. However, the reaction of the mercuric salts with urinary nitrogen compounds is so general that Liebig's titration method measured practically the total nitrogen *(36)*.

Of the "exceedingly ingenious" methods devised for the determination of urea in urine, the preferred procedure was Liebig's volumetric method *(37)*. This complicated procedure appeared in manuals during the remaining years of the 19th century. Liebig believed that the determination of urea provided a numerical measure of the metabolism in health and disease *(23)*.

Proteinuria and glycosuria, as well as uric acid, urea, and bile pigments in blood, became known as "diagnostic signs." These signs and the anticipation of finding others heightened interest in applications of chemistry to medical problems, but the expectations from Liebig's teaching, of benefit to clinical medicine, were not met. There were many isolated pieces of chemical information about blood and urine in health and disease, but they were unconnected and did not fit together anywhere. Physicians complained that chemistry was not doing anything for them at the bedside. The burst of interest and activity in the application of the simple chemical examinations of urine ended abruptly *(23, 34, 38)*. Berzelius understood

the situation when he stated in 1840 that there was a long way to go before chemical examination could differentiate between normal and diseased blood beyond the variations occurring in healthy individuals *(39)*.

This generation of physicians had received their medical training at a time when the *Naturphilosophie* doctrine of medicine with its vitalist point of view was predominant in Germany. In trying to include all natural phenomena as a whole, it was more interested in principles than in experimental details *(30)*. Physiological phenomena were not seen as mechanical processes obeying the laws of lifeless inorganic matter. Consequently, quantitative chemical and physical methods were not assigned a role in biology and medicine *(23)*. The medical concepts of this period were speculative, without close relation to medical practice. There was no place for results of chemical analyses in clinical diagnostics *(30)*. Not enough was known about basic physiology and pathology to understand the data. Analytical chemistry had outdistanced them, making the concept of a "chemical sign" premature *(34)*.

Critics and Criticisms

The belief that chemical studies were not relevant to clinical medicine was widely held and taught—even at the University of Giessen. Johann Bernhard Wilbrand (1779–1846), professor of anatomy, physiology, and natural history and a colleague of Liebig, denied chemistry the right to establish any chemical theories about physiological and pathological processes. He believed that chemistry as an empirical doctrine is, in principle, unable to produce theories to explain the process of life *(23)*. Liebig ridiculed Wilbrand as being backward and out of touch.

The Dublin clinician Robert James Graves (1796–1853) observed: "As to any benefits derived from analytical chemistry in solving the problems of vital action, or elucidating the functions of the various organs in health and disease, they may be said to be few and unimportant, and inconclusive. Few and scanty, indeed, are the rays of light which chemistry has flung on the vital mysteries." Notwithstanding all of chemistry's "boasted discoveries, we are still very little in advance of those who practised the healing art some centuries ago." Graves added that students "should never allow chemistry to cause them to absent themselves from the hospital for a single day." I "have seen students led astray by false notions, wasting half of the time which should be spent in hospital and by the sick bed, in wandering through the fields on botanical excursions, or working in the laboratory, engaged in the solution of some unimportant problem" *(40)*. Dispersed among his comments critical of chemistry in general and its changing nomenclature, are frequent pointed barbs directed at Liebig and his theories.

Armand Trousseau (1801–1867), the last of the great classic clinicians and a convinced vitalist, advised those entering medicine not to lose time "in acquiring too extensive a knowledge of chemistry." He pleaded for "a little less science, and a little more art!" He was critical "of the vanity of the pretensions of the chemists, who believe that they can explain the laws of life . . . because . . . they know the nature of some of the reactions which take place in the living body" *(41)*.

By 1883, in the preface to his book, the first of its kind in English to carry the title "Clinical Chemistry," C. H. Ralfe of London Hospital wrote: "In spite of the disparagements of such eminent clinical teachers as Graves and Trousseau, chemistry has become more and more important to the physician as a means of elucidating many pathological conditions, or of determining the character of the morbid changes effected in tissues or secretions" *(42)*.

There was also a different kind of response to Liebig's *Animal Chemistry* from the younger generation of physicians, who wanted to base medicine entirely on the natural sciences and practice "scientific medicine." Although he attacked Liebig, Carl August Wunderlich (1815–1877) saw the significance of chemistry in scientific medicine but thought it should be an auxiliary science for physicians. He rejected the idea that pathology becomes scientific by means of chemical formulae without knowledge of the diseases. He was critical of the aggressive attitude of chemistry to intervene actively in medical disputes. His criticism was directed mainly at the penetration of chemists, the "nonphysicians," into the domain of the physicians *(23)*.

Medicine was still an empirical science completely occupied with empirical observations, descriptions, and comparisons, but changes in thinking were coming. The younger physicians were turning medicine toward scientific theories and experiments and analysis of cause and effect.

The experimental logic of Liebig's theory served as the stimulus for cooperation and meaningful efforts by physiologists and chemists to seek experimental verification of his theories. Some of these investigators were Carl Schmidt (1822–1894), a former Liebig student, the physiologists Friedrich Henrich Bidder (1810–1894), and Theodor Ludwig Wilhelm Bischoff (1807–1882), a colleague at Giessen. Carl Voit (1831–1908), who later developed Liebig's metabolism theories much further, commented in 1870 that "with the hand of a master, Liebig had drawn the general outlines of the processes of nutrition" but "forgot . . . that these all are ideas and possibilities only, the validity of which had to be examined in tests on animals first" *(23)*. A variety of experimental work designed to test Liebig's claims ultimately demonstrated them to be incorrect, but the resulting thorough investigation of animal metabolism added significantly to contemporary knowledge.

Liebig, for whom physiological processes were essentially chemical, regarded the vital force as a regulator of physiological activity. In *Animal Chemistry* Liebig presented a great many metabolic reactions that were speculations derived from the formulae of the participating

compounds. Chemical speculations such as these were extremely difficult to investigate in living organs and tissues and were ultimately shown to be quite wrong. By about 1870, with the accumulation of adverse evidence, the conclusion was finally reached that Liebig's theories of animal metabolism had been discredited. Liebig's metabolic theory failed because he undervalued the importance of physiological details and had an oversimplified view of the chemical changes involved. On their part, physiologists investigating metabolic problems in vivo paid little attention to the chemical properties of the complex molecules involved *(1)*. However, Liebig's work greatly stimulated the incorporation of chemistry into physiology and clinical medicine, and his theories laid the foundations for the transition from "empirical medicine" to the new "scientific medicine" *(23)*.

Liebig vs Pasteur

When Theodor Ambrose Hubert Schwann (1810–1882) confirmed that something in addition to acidity was involved in gastric digestion (1836), he named this unknown digestive material pepsin (Greek: *pepsis,* digestion). Because a small amount was able on contact to facilitate the dissolution of a large quantity of albumin but was left unchanged throughout the reaction and did not appear in the products, he believed this to be an example of what Berzelius had recently (1835) defined as catalysis, a new force, to describe the many observations of chemical reactions, both mineral, organic, and in living matter. These reactions occurred only when some third substance was present. Liebig, who edited the journal in which Schwann's paper was published, added a footnote of caution. The terms pepsin and catalysis, said Liebig, were only representations of an idea and should not be used unless an actual substance could be shown to exist by elementary analysis *(5, 43)*.

Microscopic observations late in 1836 (published in 1837) by Charles Cagniard de Latour (1777–1859), had identified yeast as a living organism that nourishes itself at the expense of the sugar it ferments. His observations were independently confirmed at the same time by Schwann, who recognized that chemical changes take place inside the living cell, and by Friedrich Traugott Kützing (1807–1893), a botanist. However, their notion that fermentation resulted from the living nature of yeast cells was totally rejected by Berzelius, Liebig, and Wöhler. Furthermore, Liebig opposed Berzelius's concept that a catalytic force was responsible for fermentation and that the yeast was merely a nonliving catalyst. Instead of helping to clear up the mechanism of chemical reactions, it introduced a new, mysterious force into chemistry *(1, 5, 44)*.

In 1839, Liebig and Wöhler published a particularly satirical caricature of the role of yeast in alcoholic fermentation. Yeast was pictured as animal eggs that hatched with unbelievable speed into numerous small animals that devoured sugar and excreted alcohol and carbonic acid *(44, 45)*.

Shortly thereafter, Liebig came out with an entirely mechanistic explanation. Yeast, in the process of chemical decomposition, releases an albuminous substance into the sugar solution. Then, with the action of the air's oxygen on this albuminous substance, its atoms then being in violent motion, it imparts its vibration to the sugar molecules, which then break up into alcohol and carbon dioxide *(1, 2, 23, 44)*.

Liebig found himself in the middle of the debate over whether fermentation is an essential part of the life process of the yeast or a strictly chemical process of decay closely related to putrefaction. His position drew him into a famous controversy *(46)* over the "chemical" and "vital" explanations of fermentation with Louis Pasteur (1822–1895), the French chemist and bacteriologist, who held that living microorganisms are essential for fermentation. Liebig's great reputation and powerful argumentative ability gained wide acceptance among chemists for his oxygen theory of fermentation until the 1870s, when Pasteur confirmed the discovery made independently earlier by Latour, Kützing, and Schwann *(1)*.

The decisive blow to Liebig's concept was delivered when Pasteur demonstrated that yeast grows in the absence of albuminoid substances and that it ferments best in the absence of oxygen *(2, 44)*. This controversy, which began about 1857, had quieted down by 1872 with general acceptance of Pasteur's views on bacteria and the cell theory of fermentation and putrefaction. Although his discoveries in the world of microorganisms had been roadblocks to the chemical theory of enzyme action, the chemistry of life had become intimately linked to cellular physiology.

Pasteur and other microbiologists considered alcoholic fermentation to be a property of the living yeast cell, not a catalytic process, and required the presence of a living organism. For Pasteur the living organism was the ferment *(44)*. Although he was mistaken, his work stimulated the rapid development of medical microbiology.

Summary

Liebig achieved prominence in general analytical, inorganic, organic, and agricultural chemistry and in the broad field of animal physiological chemistry. Few of his physiological theories were highly original, none of them were definitive, and many were later shown to be incorrect. However, his stature should be measured by his participation at a crucial stage in the development of chemistry, mainly his role in the development of organic chemistry between 1829 to 1839.

Among Liebig's lasting contributions to organic chemistry were the analytical methods he devised or refined, the many compounds and reactions he discovered or described, and the quantitative techniques and apparatus he designed to overcome difficult operations and sources of error in determining the composition and reactions of

physiologically important compounds. He demonstrated how the quantitative methods of organic chemistry can be applied to the investigation and study of all the phenomena of the animal body and thereby provided one of the first comprehensive pictures of the overall unending chemical exchanges that form an integral part of the vital processes. Through the reliability of his analyses, the thoroughness of his examination of the products and reactions of every chemical problem, Liebig's influence is felt today through the work of the many scientists he trained to think clearly and logically and to experiment intelligently and accurately in gathering the facts. *Animal Chemistry* had a significant impact on the future course of physiological thought and investigation by the discussion and new research by others that his work helped to stimulate.

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