LOUIS RENÉ LE CANU

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RESUMEN. Louis René Le Canu (1800-1871), médico y farmacéutico francés que dedicó la mayor parte de su vida a estudios fisiológicos de la sangre y de la orina, así como a la destilación de una variedad de aceites vegetales, en particular, el aceite de ricino.

ABSTRACT. Louis René Le Canu (1800-1871), French physician and pharmacist, who dedicated most of his life to the physiological examination of blood and urine, and the distillation of several vegetable oils, in particular, castor oil.

Life and career[1-3]

Fig. 1*. Louis René Le Canu (1800-1871).*

Louis René Le Canu (Figure 1) was born in Paris on November 18, 1800, the son of Marie-Thérèse Delondre and Jacques Louis Toussaint Le Canu, a former chief pharmacist of the Salpêtrière and of the General Hospitals of Paris. He took his basic education at the Lycée Charlemagne and after receiving his baccalauréat ès lettres he entered the chemistry laboratories of André Laugier (1770-1832) and Louis Nicolas Vauquelin (1763-1829) at the Muséum d'Histoire, Naturelle, to follow the career of his father. After two years, he transferred to a position of préparateur at the chemistry laboratory of Louis-Jacques Thenard (1777-1857) at the Collège de France, where his clear abilities brought him to become superintendent of chemical operations and a close friend of Thenard. After three years with Thenard he began his studies at the École de Pharmacie from where he graduated as pharmacien de 1re classe. In 1832 he was appointed adjunct professor at the École de Pharmacie de Paris and in 1833 promoted to Professeur titulaire de Chimie et Pharmacie. At the same he followed studies at the Faculté de Médicine de Paris, from where he graduated in 1837 after successfully defending a thesis about the chemistry of human blood.[4] At an early life he retired into country life and devoted himself to agricultural research.

In 1828 Le Canu married Adéline (?-1871), the daughter of Antoine Germain Labarraque (1777-1850), a well-known chemist and pharmacist; two daughters were born of this union. Le Canu passed away in December 19, 1871, after a strong attack of pneumonia.

Le Canu received many awards and honors for his scientific achievements and public activities. He was elected member of the Conseil d'Hygiène Publique, of the Conseil de Salubrité de la Seine (1830) and Vice President of the same (1844), member of the Académie de l'Industrie (1831), member of the Institut Historique (1834), member of the

Société Philomatique (1834), and associate member of the Société d'Émulation (1856). He was nominated officier of the Légion d'Honneur (1860), chevalier of the Ordre Royale de Charles III of Spain (1860), chevalier of the Ordre Royale of Villaviçosa, Portugal (1862), commander of the Order of Santa-Rosa y de la Civilización de Honduras (1870), etc. He was also elected to many French and foreign scientific societies, among them: member of the Académie de Médicine (1838), corresponding member of the Société des Sciences Naturelles de Cherbourg (1853); corresponding member of the Colegio de Farmacia de Madrid (1856), corresponding member of the Académie de Médicine de Poitiers (1858), corresponding member of the Académie des Sciences de Lisbon (1858), etc.

SCIENTIFIC WORK

Le Canu wrote more than 175 papers, booklets, and books (for example[5-11]), particularly about the chemistry, composition, and properties of blood and urine, in the normal and pathological states, organic, inorganic, and analytical chemistry, vineyard problems and wine production, etc. As customary for all candidates to the Faculté de Médicine, he published a booklet describing his researches and achievements.[12] In addition to the subjects discussed below Le Canu studied formic acid and its formiates,[13] the presence of succinic acid in turpentine;[14] succinic and benzoic acids,[15] the preparation of uranium oxide;[16] the composition of the Chéronie zinc sulfide mineral,[17] uranium,[18] the analysis of hermodacte,[19] opium medications,[20] the falsification of flours,[8] grapes, their products and vinification;[11,21] etc.

The publication about the falsification of flours[8] earned Le Canu a prize from the Société d'Encouragement (1850).

The composition of blood

Le Canu carried extensive research on the composition and properties of blood.[4,5,9,22-28]

His first publication was devoted to the separation and study of the physical and chemical properties of the coloring matter of blood, which Chevreul had named *hematosine*.[5,29] In the introduction Le Canu wrote that previous researchers had attributed the color of blood to a variety of factors (such as an animal substance of particular nature, to iron present in a particular state of combination, etc.), had developed different methods for isolating it, and reported contradictory information about its properties. Le Canu believed that these inconsistencies originated from the fact the isolated hematosine was mixed with small amounts of albumin, serum, or fibrin, or in a state that made it insoluble in water. For this reason he went on to develop a better separation procedure based on the known fact that evaporation of a solution of coloring matter at a temperature below 50 ºC, recovered it in a soluble state. Accordingly, he first separated a certain amount of blood clot of beef in very small parts, diluted them in distilled water to separate the entrained serum, expressed the resulting mass, and repeated the procedure several times to eliminate the accompanying fibrin. The final filtrate, left to evaporate spontaneously under the action of sun, yielded the coloring matter in a very pure state.[29]

Le Canu reported that the coloring matter was a bright jet black solid when in mass, dull and brick colored when powdered, and bright, red, and transparent, when in thin layers. Cold water dissolved it easily, producing a red solution having a faint flavor and odor, which persisted for a long time. The solid substance, heated to above 66 ºC, lost its color and turned into a flocculent material completely insoluble in water. Le Canu prepared aqueous solutions of equal concentration of the coloring matter and of albumin, subjected each to the action of heat, red litmus paper, alcohol, solutions of chlorine, nutgalls, lime, ammonia, acetic acid, HCl, nitric acid, sulfuric acid, potassium, ferric ferrocyanide, mercuric chloride, potassium iodide, and lead acetate, and tabulated the results. Some of them were as follows: (a) the hematosine solution did not affect litmus paper; that of albumin turned it blue; (b) alcohol produced a red and a white precipitate, respectively; (c) both solutions produced a white precipitate with HCl and did not affect ferric ferrocyanide, potassium iodide lead acetate, or limewater; (d) nut galls, nitric acid, and sulfuric acid produced a brown precipitate with the coloring matter, and a white one with albumin. Both precipitates were insoluble in an excess of acid, etc. All the experimental results indicated that hematosine was completely different from albumin and contained a very large amount of iron.[29]

Le Canu also reported the preparation of globulin from beef blood and a comparison of its properties with those of albumin. The results indicated that globulin differed from albumin by its color, by the large amount of iron it contained, by being high soluble in alkalis and acids, and its property of forming with HCl a compound soluble in alcohol.[29]

The following publication[22] was divided in three parts; in the first one Le Canu described the numerous immediate principles he had identified in blood, as well as their properties and proportions; in the second part he reported a comparative analysis of the blood of individuals in different states of health, and in the last one he discussed in particular the composition of the blood of individuals affected by jaundice.

For the first part of his work Le Canu precipitated fresh human venous blood with a large excess of concentrated alcohol to be sure that the resulting liquid remained transparent. The precipitated flakes were filtered through a piece of cloth and then treated several times with boiling alcohol to separate the soluble from the insoluble components. The alcoholic extract was evaporated to dryness in a water bath and then extracted with ether. Evaporation of the ethereal extract produced a red brown residue having the consistency of turpentine, which seemed to be composed of two substances, one a pearly solid, the other an oily liquid. The solid phase was extracted and recrystallized with alcohol several times, and eventually it crystallized as white pearly plates, without odor and taste, melting at about

150 °C and looking very similar to cholesterol. Further heating decomposed it into ammonia products and a residue containing phosphorus. The oily phase had an acrid taste, was insoluble in cold and hot water, and soluble in ether and alcohol. The resulting solution had no action upon litmus paper. Potassium hydroxide converted this substance into an acid one. All its properties indicated that it was a true immediate principle of blood.[22]

Le Canu treated the substance insoluble in alcohol with a variety of reagents (ether, inorganic acids, ammonia and KOH, lead sub-acetate, etc.) and concluded that it contained fibrin, albumin, coloring matter, and a variety of soluble salts, such as potassium and sodium chloride, carbonate, and phosphate. Analysis of the liquid fraction of blood (serum) indicated that it contained over 90% of water, 7.8 to 8.1% albumin, 0.17 to 0.20 % organic matters soluble in alcohol, 0.2 to 0.26% albumin combined with sodium, 0.1 to 0.2% fatty matters crystallizable, and about 1% of different inorganic salts. Le Canu also reported the amount of fibrin present in the blood of 22 different human beings affected by different illnesses.[22]

His final results indicated that 1000 parts of blood contained 780.145 to 785.590 parts of water, 2.100 to 3.565 of fibrin, 65.090 to 69.415 of albumen, 119.626 to 133.000 of coloring matter, 2.430 to 4.300 of crystallizable fatty matter, 1.310 to 2.270 of oily matter, 1.790 to 1.920 of extractive matter soluble in alcohol and water, 1.265 to 2.010 of albumin combined with sodium, and 8.718 to 10.470 of inorganic salts. The losses in the analysis varied between 2.400 to 2.586 parts.[22]

In the second part of this publication Le Canu reported the average composition of the blood of ten males and 10 females and tried to deduce some possible physiological consequences of the differences observed, according to sex, age, and temperament (e.g. sanguineous and lymphatic). The basic experimental procedure consisted in drying the serum, separating from the residue the soluble salts and extractive matter by means of boiling water, and, finally drying the clot and determining its composition. He summarized the results in a series of tables. The first two reported the quantities of water, albumen, soluble salts, and globules, and temperament, for each sex. The following two tables reported the relative proportion between serum and globules, the proportions of water, soluble salts and extractive matter, albuminous substances (fibrin and albumen), the amounts of water and suspended substances, and the proportion between water and fixed substances. The last table reported the analysis of the serum of the 20 individuals.[22]

Le Canu concluded as follows: (a) the serum content varied according to sex and age. Females had a larger proportion of serum than males and lymphatic individuals more than sanguineous ones; (b) the content of albumen, fibrin, and coloring matter varied according to sex and age. Within individuals of the same sex but different age, it was less in females than in males. Within the same sex, it was larger for lymphatic individuals; (c) differences in nutrition and age did not did not reflect in differences in blood composition for individuals of the same sex; (d) the ratio of albumen and water present in the serum changed with sex and age; and (e) the menstruation fluctuation affected the proportion of globules.[22]

In 1831 the Académie Royal de Médicine established a competition for the best work that would provide a comparison between the composition of the blood of people suffering from jaundice, and that of normal blood, as well as establishing the chemical differences. Most physicians believed that the characteristic yellow color of people suffering from jaundice was due to the presence of bile in the blood, although no experimental evidence had been provided for this claim. Henri Braconnot had established that bile contained a particular resin, oleic and margaric acids, cholesterol, an animal matter (containing nitrogen), a bitter principle, another sweet, a small amount of a coloring matter, which Chevreul had separated into three colored components, one yellow orange, another slightly blue, and another green.[30]

Le Canu examined a venous blood sample, which had been taken about 36 hours before from a patient suffering from jaundice and acute pneumonia, and was already covered by a strong skin. He separated and analyzed the different parts of this sample, as well as samples of normal blood taken from a male and a female. His results indicated that the blood of jaundice patients, in addition to fibrin, albumen, coloring matter, and soluble and extractive matter, also contained jaundice patients (1) extractive matters, soluble in alcohol and water, (2) a soluble combination of albumen and sodium (serosity), (3) an insoluble combination of albumen and sodium, (4) a crystalline fatty substance, (5) an oily matter, (6) a yellow orange principle combined with the oily matter, and (7) a blue coloring principle. His results clearly proved the presence of bile components in the blood of jaundice patients.[22]

This publication earned Le Canu a gold medal worth 500 francs from the Académie Royal de Médicine and the Monthyon Prize from the Académie des Sciences (a medal worth 1,500 francs).

In an additional paper Le Canu reported that the blood of cholera patients was more consistent, contained a larger quantity of fixed matter and much less water than the blood of normal persons. No particular changes were noted in the amounts of fibrin, albumen, and coloring matter.[23]

Le Canu summarized his extensive research on the composition of blood in normal and pathological situations in a thesis he presented to the Faculté de Médicine de Paris for obtaining the degree of doctor of medicine.[4,25,26] The thesis was divided into four sections, more or less according to the explanations given above. The first one described the number, the nature, and arrangement of the first principles (at least 25 in number) present in venous blood; the second, the quantitative determination and relative proportion of these principles, as a function of sex, age, temperament, and food regime; the third section, provided a comparison between venous and arterial blood, and the fourth, the pathological changes of blood as a consequence of certain diseases. Each section contained a detailed

explanation of the pertinent experimental and analytical procedure. Venous blood of the hand was selected as prototype because it represented the simplest state. Among the substances present in blood Le Canu mentioned water, oxygen, nitrogen, CO_2, iron, sodium, potassium and ammonia chlorides, potassium sulfate, sodium, calcium, and magnesium bicarbonates and phosphates, fixed fatty acids combined with sodium, sodium lactate, fatty phosphorescent matter resembling cerebral matter, a salt of a volatile fatty acid, cholesterol, seraline, fibrin, albumen, yellow and red coloring matter.[4]

Le Canu expressed his findings in 25 propositions, for example: (a) the red coloring matter was not a true immediate principle, it was the product of chemical reactions or a mixture of coloring matter and albumen; (b) independently of the method of separating its, this coloring matter was always combined with iron in a very intimate form; (c) the coloring principle had the same properties in mammals, birds, reptiles, and fish; (d) after coagulation, the serum contained in a dissolved state all the blood components excepting part of the fibrin, hematosine, and albumen. The remaining fibrin, hematosine, and albumen was present in the clot; (e) arterial blood, compared to venous blood, contained less water, more globules, less carbon, much more oxygen combined, and much more free oxygen than free carbon dioxide; (f) the blood of patients affected by diabetes, typhus, and heart disease, contained less globules and more water than normal blood; etc.[26]

Urine

Le Canu published several papers about the presence of urea in urine and its analysis.[6,31-33] In his first paper on the subject he wrote that having been commissioned to analyze a urine sample of a female affected by dropsy, he had tried to determine the urea content using the procedure reported by Étienne Ossian Henry (1798-1873) but failed to obtain satisfactory results.[31] Henry's procedure consisted in mixing fresh urine with a slight excess of lead sub-acetate and concentrating the liquor slightly acidified with sulfuric acid until crystallization.[34] Additional trials using the older method proposed by Antoine François Fourcroy (1750-1809) and Louis Nicolas Vauquelin (1763-1829) had also been unsuccessful. The Fourcroy-Vauquelin procedure consisted in evaporating the urine to syrup and left to crystallize by cooling. Extraction of the residue with concentrated alcohol separated the urea from the mineral salts. The alcoholic extract was again evaporated to syrup and left to crystallize by natural cooling into impure urea.[35]

Le Canu believed that the failure was due to an incomplete separation of the urea from the other salts present in the liquid, and its possible decomposition during the evaporation steps. In a brilliant decision he decided to improve the process by using the fact that Fourcroy and Vauquelin had reported that urea and nitric acid seemed to form a salt of definite composition, insoluble in an excess of acid. Hence, this salt could be isolated from the urine and used to determine the amount of urea present in the original fluid. He first prepared urea nitrate by adding nitric acid to an aqueous solution of urea, followed by separation of the precipitate and purification by recrystallization from distilled water to eliminate the excess of acid. A given amount of the dry precipitate was then dissolved in an aqueous solution of sodium bicarbonate containing 50 g of the salt per 400 g of distilled water. The result of all these operations indicated that 100 parts of urea nitrate contained 46.50 g of nitric acid and 53.50 g of urea.[31]

The improved analytical procedure consisted in evaporating the urine sample to reduce its volume by about 10%, followed by addition of nitric acid, filtration drying, and weighing the urea precipitate.[31]

Le Canu used his analytical procedure to prove that the kidney's liquid contained urea.[32]

In a following paper Le Canu wrote that although human urine had been the subject of a large number of publications and the identity of its main components had already been established, no one had taken into detailed consideration the possibility that its relative composition varied with the time of the day, the sex, age, health situation, nutrition regime, etc. It could well be that these possible changes explained the contradiction present in the information published. He now wanted to report the results had had obtained on this subject, using 16 individuals of the following ages and sexes: (a) 6 men aged 20 to 45 years, 2 old men aged 84 to 86 years, 4 females aged 18 to 18 years, and 4 young children. In every case, he had measured the amount of urine passed during 24 hours, its density, and content of urea, uric acid, fixed substances, inorganic salts, etc.[6]

The results of all these measurements indicated that (i) the amount of urine passed during 24 hours by the 16 individuals varied from 525 to 2271 g; (ii) urine had the highest density in men and in early life; (iii) the average amount (in grams) of urea yielded in a day, measured during 12 days, varied as follows: men 28.05; women 19.11; old persons 8.11; children of 8 years 13.47, and children of 4 years 4.50; (iv) at the same time the production of uric acid was also very variable: A man of 20 years produced 0.995 g, a man of 22 years 0.997 g, a man of 38 years 1.120 g, a girl of 19 years 0.472 g, and a woman of 43 years 0.454 g, (v) the average amounts of salts (g) were men, 16.88, women, 14.38, children, 10.05, and old persons, 8.05; (vi) the amount of sodium chloride varied greatly, according to the nature of the food, and individual peculiarities; (vii) men in the prime of life secreted most solid matters, women less, and old persons and children least of all; (viii) during equal periods of time, a given individual secreted equal amounts of urea and of uric acid, (ix) during equal periods of time, different individuals secreted different amounts of urea and uric acid, (x) the variable quantities of urea, secreted by different individuals during equal periods of time, were proportional to the age and sex of the persons. It was greater in men than in women, and in women than in old persons and children; (xi) the amounts of earthy phosphates passed by different individuals during 24 hours varied between 0.029 and 1.960 g and were not related to the sex or age of the individual; (xii) the amounts of sulfuric acid secreted in the form of sodium and potassium sulfate by different individuals during 24 hours varied between 0.988

and 3.730 g; and (xiii) the amounts of phosphoric acid secreted in the form of sodium and ammonium sulfate by different individuals during 24 hours varied between 0.011 and 1.843 g.[6]

According to Le Canu, the large variation among the amounts of most of the components of urine clearly demonstrated the caution to be taken when describing the constitution of urine.[6]

In his last paper on the subject Le Canu addressed the question of on which state was urea present in the urine. Three different answer had been provided: (i) urea did not pre-exist in urine, it was generated from other principles, under the influence of heat; (ii) urine did not contain urea in its final form but as the chloride of a radical (*uryl*) composed of 2 atoms of nitrogen and 4 of hydrogen; and (iii) it was present in urine as its lactate. Le Canu carried four series of experiments; in the first one he exposed the urine of several healthy individuals during 18 to 24 hours to temperatures between 11 ° and 12 °C, withdrawing every so often the remaining liquid. In every opportunity, addition of nitric acid or oxalic acid led to the formation of pearly plates of urea nitrate or urea oxalate, respectively. Urea mixed with nitric acid, without the influence of heat, always produced the same plates of urea nitrate when cooled down. In the second series of experiments Le Canu evaporated urine in a water bath to the consistency of syrup and then diluted it with commercial alcohol. On evaporation and cooling, the alcoholic solution deposited a solid mass composed essentially of alkaline sulfates, chlorides, phosphates, and lactates, accompanied by small amounts of ammonium chloride and urea. Further treatment transformed this mass into another, acid and deliquescent, having the consistency of honey and very soluble in alcohol at room temperature. This residue was found to be composed of two substances, one that regenerated lactic acid, potassium lactate, urea, and ammonium chloride; another, white, unaffected by air, volatile without blackening while producing white vapors composed mainly of ammonium chloride and carbonate, and leaving a residue containing traces of sulfate, chloride, phosphate, and alkaline carbonates. It was soluble in alcohol, cold or boiling, and very soluble in cold distilled water. Treatment with nitric acid precipitated urea nitrate; with silver nitrate, a precipitate of silver chloride, and becoming colored blue with a mixture of cupric sulfate and KOH. All these results identified it as urea mixed with small amounts of ammonium chloride, and alkaline sulfate, chloride, phosphate, and lactate.[33]

Two additional series of experiments led Le Canu to conclude as follows: (i) the extraction of urea by thermal means were not the consequence of the production of urea by these means; (ii) the assumed uryl chloride was merely an intimate mixture of the combination of urea with ammonium salt; (iii) the assumed urea lactate was basically a mixture of urea with free lactic acid, (iv) urea could be extracted in a very pure form by means of alcohol, without the help of acids or alkalis; and (v) urea was present in urine in a free state.[33]

This work won Le Canu an honorable mention in the 1841 competition on physiology sponsored by the Académie des Sciences.

Fatty materials

Le Canu, alone or with Antoine Alexandre Brutus Bussy (1794-1882) and José Luis Casaseca (1800-1869), published a series of papers about their research of fats, fatty oils, fatty acids, and their derivatives.[36-44]

In 1826 Casaseca and Le Canu reported the presence of oleic and margaric acid (palmitic acid) in the hulls of Levant (Levant or Indian berry, Moonseed, *Menispermum cocculus*).[44] It was known that boiling these hulls released a large amount of fatty substance, which on cooling solidified as a slightly colored matter. Repeated washings with distilled water completely removed the color and left a residue having all the external characteristics of tallow, except that when wet, it reddened litmus paper. Treated with boiling concentrated alcohol it produced a green extract, which also reddened litmus paper and on cooling precipitated white flakes. Repeated dissolution and crystallization led to a completely neutral substance. The acid component was retained by the alcoholic extracts. Evaporation of the latter left an acid residue, solid at room temperature, which pressed between filter paper, provided a colorless pearly solid, fusing at 59 °C, partly soluble in cold alcohol, very soluble in boiling alcohol, and having all the properties of palmitic acid. The liquid phase retained by the paper was identified as oleic acid. Further analysis of the whole hulls showed that they also contained stearin.[44]

In their first paper about the distillation of fatty materials Bussy and Le Canu reviewed the available knowledge, particularly the one reported by Michele Chevreul (1786-1889) and Louis-Jacques Thenard (1777-1857).[37] Chevreul had extensively studied the preparation and properties of animal and vegetable fatty materials,[45] although he had paid little attention to the products formed during their distillation. It was assumed that the distillation of fatty materials yielded water, acetic acid, methane, carbon dioxide, a particular acid that Thenard had designated as sebacic acid (decanedioic acid),[46] and a large amount of fatty material, sometimes liquid, sometimes solid, of unknown nature and composition.

Bussy and Le Canu decided to investigate this latter fraction in more detail and for this purpose they distilled a large number of fatty materials of animal and vegetable nature (e.g. tallow, pork fat, oil of olives, poppy seeds, bitter almonds, linseed, etc.). Their results indicated that the distillation of a fatty material followed three distinct stages, characterized by the materials that passed over. The first fraction contained large amounts of oleic acid and margaric acids accompanied by a gas composed of flammable gases mixed with methane, carbon monoxide, and carbon dioxide. It was followed by another fraction composed of empyreumatic oil exempt of fatty acids. After all the distillates had passed, a yellow red substance sublimed. Although the proportion of these substances varied according to the nature of the raw material being distilled, the general characteristics of the process were the same, as illustrated

by the heating of poppy seed oil: At about 200⁰C a mucilaginous flaky substance began depositing while the oil became colorless. Once the oil started boiling, gases were released; the oil achieved a strong penetrating odor and then began distilling without becoming colored. The first third distilled was a liquid that on cooling turned into a soft solid; afterwards the remaining oil became odorless odor and passed over as a non-acid liquid, leaving in the flask a carbonaceous residue. According to Bussy and Le Canu 100 g of oil yielded about 4 to 5 liters of gas, 1 to 2 g of carbon, and 92 to 94 g of distillate.[37]

Bussy and Le Canu analyzed the different fractions distilled and found that the first one solidified at about 20⁰C into it a yellow and very odorant soft material, completely soluble in alcohol, which reddened litmus and combined with an aqueous solution of KOH forming a real soap. This substance was found to be a mixture of acetic, sebacic, oleic, and margaric acids, empyreumatic oil, and smelly oil; it was quite similar to the product that Chevreul had found when distilling stearin and olein. The sebacic acid was easily separated by means of boiling water; the remaining semi-solid mass was pressed between sheets of papers and separated into a compact colorless pearly mass of margaric acid, melting at 57⁰C, and a liquid found to contain oleic acid. The remaining fractions distilled did not contain substances of particular interest.[37]

In a following paper Bussy and Le Canu reported their test of the fact reported in the literature that treatment of lard and other fatty materials with nitric acid of density 1.500 transformed them into substances having a higher melting point, allowing their use in the fabrication of candles.[38] For this purpose they treated lard during one hour with four times its weight of concentrated and boiling nitric acid. Afterwards the mixture was allowed to cool and the fatty matter, which floated upon the liquid, was separated. This yellow inodorous matter was softer than the original lard and almost completely soluble in alcohol. The residue appeared to be fat, probably a little altered. The alcoholic extract reddened litmus paper strongly; by evaporation in a water bath it gave a yellowish residual mass, which was then pressed between folds of blotting paper. Inspection of the solid and liquid phases showed they were composed of palmitic and oleic acids, respectively. These results indicated that nitric acid was able to partly convert fat into oleic and palmitic acids, a conclusion that Bussy and Le Canu believed could be extended to all fatty bodies consisting of stearin and olein; a result already found for the alkalis, sulfuric acid, oxygen, and heat.[38]

In a following publication Bussy and Le Canu extended their distillation experiments to fatty materials such as blubber oil (spermaceti), which could be saponified in spite of not containing stearin and olein, and ethal (cetylic alcohol) and the crystalline matter of gallstones, which were not attacked by alkalis.[39,41]

Chevreul had reported that spermaceti was a substance hardly saponified by alkalis, which on long contact transformed into (a) a substance soluble in water (*éthal*), which did not have the sweet flavor of the principle (glycerin) present in oils, (b) a fatty acid congener to margaric acid, and (c) another fatty acid congener to oleic acid.[47] These results led Bussy and Lecanu to believe that spermaceti could also be transformed by distillation into the same fatty acids produced by saponification. Their results of their experiments proved this assumption to be true: distillation of crude spermaceti proved that it contained spermaceti, colorless oil, oleic, margaric, and acetic acids, water, odorous substance, a yellow material, and yellow empyreumatic oil. In addition, it did not contain the sebacic acid and the volatile oil present in fats composed of stearin and olein.[39,41]

Upon distillation, cholesterine (cholesterol) passed over almost completely, leaving little residue; the distillate had all the properties of the original material, contained no fatty acids and gave no reaction with litmus paper. Similar results were obtained with ethal.[39,41]

These results suggested that distillation and the action of alkalis could be used to distinguish between fatty and analogous bodies[39,41] and for this reason Bussy and Le Canu decided to extend their experiments to castor oil and obtain additional information regarding its nature.[40] Their results indicated that castor oil reacted in a very particular manner to the action of heat and alkalis and differed completely from the vegetable oils composed of olein and stearin. Distillation of castor oil did not produce a solid product composed of oleic and margaric acids, followed by a distillate of empyreumatic oil and a yellow solid residue; now the products were completely different: initially the oil distilled without release of gases, followed by a mixture of volatile and fixed oils, acetic acid, and fixed fatty acids. Gases were released after about one-third of the charge had passed over. Afterwards, the residue began to thicken and swell until most of the retort became filled with a yellow spongy and elastic substance. The latter was odorless and tasteless, slightly sticky, insoluble in fixed and volatile oils, alcohol, and ether, even at their boiling point. It was easily dissolved in alkalis, producing a soap having all the properties of ordinary soap, and was little affected by sulfuric, nitric and hydrochloric acids.[40,42]

The volatile oil was colorless and very transparent; had density 0.815, began boiling at about 100 ºC and continue to do so up to 150 ºC. This result indicated that it contained at least two different substances. After prolonged cooling it turned into a solid mass composed of prismatic crystals. The fatty material accompanying this oil was found to be a mixture of two new fatty acids, one solid, the other liquid, which Bussy and Le Canu named *ricinic* (today, ricinoleic acid), and *elaiodic* respectively. Ricinic acid was a white pearly acrid solid, melting at 22 ºC, completely insoluble in water and very soluble in alcohol and ether. On heating it volatilized without alteration and combined with salifiable bases; with KOH and NaOH it formed salts very similar to margarates (palmitates), which were soluble in water and in alcohol. Elemental analysis indicated that it contained, by weight, 73.56% carbon, 9,86% hydrogen, and 16.58% oxygen. Elaiodic acid remained liquid even at temperatures below 0 ºC; it was acrid and completely soluble in alcohol

and ether. Its combinations with NaOH and KOH were very soluble in water and in alcohol, while those with magnesia and lead oxide were soluble in alcohol, from where they crystallized as white oily needles.[40,42]

Bussy and Le Canu found that saponification of castor oil generated not only glycerin and ricinic and elaiodic acids, but also a clearly new acid, which they name *margaritic acid* (today ricinostearic acid). Margaritic acid was a white pearly solid, odorless and tasteless, insoluble in water, slightly soluble in alcohol, and containing, by weight 70.50% carbon, 10.90% hydrogen, and 18.60% oxygen. It strongly reddened litmus paper and with salifiable bases formed salts analogous to margarates.[40,42]

Bussy and Le Canu concluded that the main products of the distillation of castor oil were an oil, volatile between 100 º and 150 ºC, a particular solid substance, which constituted the residue of the process, and ricinic and elaiodic acids. These substances were not present in castor oil, they were the result of the distillation process.[40,42]

Bussy and Le Canu summarized their results in a book published in 1829.[43]

A further paper by Le Canu discussed the existence of cholesterol in yolk.[36]

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