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REVISION BIBLIOGRAFICA

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ABSTRACT

Jules Lefort (1816-1889) was a French pharmacist and chemist who did extensive research on toxicology and forensic tests, particularly for morphine and phosphorus. He studied the coloration produced by different reagents with morphine, narcotine, brucine, and strychnine, and the chemical and toxicological effects produced by morphine during its passage through the animal economy. Morphine resisted without alteration the most active and extended putrefaction of animal matter. Salts of iron sesquioxide were the best reagent for assuring the presence of morphine in powder or concentrated solution; iodic acid with ammonia could detect morphine in a liquid containing 1/10,000 part of the alkaloid, and morphine ingested in a continuous manner and in variable doses, would appear in the urine but not in the sweat. He proved that HCl gaseous was the best reagent for detecting the presence of digitaline in colorless liquids and that this compound could be neatly separated by dialysis. Leroy reported the differences between the ipecacuanhas originating from Brasil and from New Granada; the latter contained 23% less emetine. He also developed a very efficient procedure for the separation of this principle. Leroy found that the odor released by putrefying bodies, as well as their slight emission of light, were caused by a sulfur derivative and a combination of hydrogen and phosphorus. He also determined the amount of atropine present in the leaves and roots of belladonna and proposed an efficient method for its extraction.

Keywords: atropine, digitaline, forensic tests, morphine, phosphorus, toxicology.

RESUMEN

Jules Lefort (1816-1889) fue un farmacéutico y químico francés que se dedicó a la toxicología y ensayos forenses, especialmente de la morfina y el fósforo. Reportó la coloración generada por diversos reactivos con la morfina, narcotina, brucine y estricnina, así como los efectos químicos y toxicológicos producidos por la morfina durante su pasaje por la economía animal. La morfina resistía sin alteración la putrefacción más activa y extensa del material animal. Las sales del sesquióxido de fierro eran el mejor reactivo para asegurar la presencia de morfina en polvo o en soluciones concentradas. El ácido yódico con amoníaco era capaz de detectar morfina en un líquido conteniendo 1/10.000 partes de alcaloide, y la morfina, consumida en forma continua y en dosis variable, aparecía en la orina y no en el sudor. Demostró que el HCl gaseoso era el mejor reactivo para detectar la presencia de digitalina en un líquido incoloro y que este compuesto podía ser separado en forma nítida por diálisis. Leroy reportó las diferencias entre la ipecacuanha proveniente de Brasil y la de Nueva Granada; esta última contenía 23% menos de emetina. También desarrolló un proceso muy eficiente para separar este principio. Leroy encontró que el olor desprendido por los cuerpos en putrefacción, así como su débil fosforescencia, se debían a un derivado sulfurado y a una combinación de fósforo e hidrógeno. También determinó la cantidad de atropina presente en las hojas y las raíces de la belladona y propuso un método eficiente para su extracción.

Palabras clave: atropina, digitalina, test forenses, morfina, fósforo, toxicología.

INTRODUCTION

Life and career (Lefort, 1871c)

Very little information is available about the life and education of Jules Lefort (1816-1889). After finishing his basic education, he won by competition a position as intern student in the civil hospitals of Paris (1841). His brilliant studies earned him the First Prize (silver medal) of the 1844 competition of the Internat in pharmacy of the Parisian hospitals. In 1845 he was awarded his diploma of Pharmacien de 1er classe and in 1856 the prize of the Académie des Sciences for the best work on mineral waters. In 1856 the French Academy of Medicine awarded him a silver medal for his book on hydrological chemistry and in 1860 the French Academy of Sciences bestowed him an honorary mention for his memoir about glucose, done in collaboration with Jean Léonard Marie Poiseuille (1797-1869). In 1861 the Academy of Medicine awarded him the Capuron Prize for the publication of his dictionary about mineral waters, in collaboration with the physicians Maxime Durand-Fardel (1815-1899) and Louis Eugène Le Bret. In 1864 he was appointed Chevalier of the Legion d'Honneur. Leroy served as vice president of the Société d'Hydrologique Médicale de Paris (1870) and President of the Société de Pharmacie de Paris (1871).

Scientific contribution

Lefort published about 80 papers and books (i.e., Lefort, 1855, 1871b, 1873) in the areas of inorganic and organic chemistry, hydrology, physiology and physiological chemistry, toxicology, etc. He also published a booklet describing his research and achievements, as customary to candidates to the Académie Impériale de Médecine de France (Lefort, 1871c). In addition to the subjects described below, Lefort also studied the reaction of halogens with fatty acids (Lefort, 1852, 1853); the composition and nutritive value of edible champignons and truffles (Lefort, 1856, 1857); the presence of glucose in the liver of fish and a large number of animals (Poiseuille & Lefort, 1858); demonstrated the presence of urea in the milk of herbivore animals (Lefort, 1866a); studied the properties and uses of the seeds of buckthorn (*Nerprun tinctoria*) (Lefort, 1866b, 1868c); the composition and properties of humus (Lefort, 1867a); the preparation and use of tar water in medicine and tinctures (Lefort, 1868ab), the contamination of the water of artesian wells, caused by the vicinity of cemeteries (Lefort, 1871a); the toxicology of phosphorus (Lefort, 1874b); the influence of Arabic gum on certain chemical reactions (Lefort & Thibault, 1882); etc. His contributions to inorganic and organic chemistry will be discussed en another publication.

Alkaloids

Coloration phenomena

In his first paper about alkaloids, Lefort wrote that morphine, narcotine, brucine and strychnine were easily identified by means of the typical colors that their solutions produced in contact with certain reagents (Lefort, 1844b). In 1836 Jean Pierre Couerbe (1805-1867) described the color reaction that took place between narcotine and a mixture of sulfuric and nitric acids (Couerbe, 1836) and in 1840 J. B. Berthemot suggested the use of brucine acidulated with sulfuric acid for recognizing the presence of small amounts of nitric acid (Berthemot, 1840). In addition, morphine hydrochloride, morphine acetate, and commercial strychnine were known to turn red under the action of nitric acid. In 1830 Sérullas suggested using iodic acid to detect the presence of morphine (Sérullas, 1830), but in 1839 W. Davidson proved that certain urines and all the liquids from the stomach decomposed iodic acid (Davidson 1839). This information led Lefort to investigate the possibility that these colorations intensified under the influence of sulfuric acid (Lefort, 1844b). His first results indicated that diluted solutions of these four alkaloids did not develop a red color when treated with nitric acid but did upon addition of a few drops of sulfuric acid. These colorations were so similar that they could not be used to differentiate between the alkaloids. Lefort wrote that it was easy to predict that sulfuric acid in contact with a vegetable alkali and an oxidant like nitric acid led to decomposition of the latter. He tested the coloring ability of nitric acid, alone or mixed with sulfuric acid, and reported the following results: (1) diluted solutions of brucine, morphine and its salts, and impure strychnine were not colored by nitric acid but did upon addition of a few drops of sulfuric acid; (2) a few centigrams of morphine, its hydrochloride or acetate, mixed with sulfuric acid and most iodates, produced intense red colorations while depositing iodine; potassium antimonate and sulfuric acid produced a pale red color, and lead dioxide and sulfuric acid a red one; (3) narcotine did not redden with a mixture of nitric and acetic acids. It did with a mixture of sulfuric acid with iodic acid, most of the iodates, and lead dioxide. Chloric acid, potassium chlorate, potassium perchlorate, chlorous acid, and lead or potassium chlorites produced the same result; (4) brucine was reddened by nitric acid, by a mixture of iodic acid and by most iodates with sulfuric acid, by a mixture of sulfuric acid with chromic acid, potassium chromate, potassium dichromate, chloric acid, most of the chlorites and chlorates, and a mixture with lead dioxide, potassium antimonate, or potassium nitrite; (5) pure strychnine did not react with nitric acid but commercial strychnine turned red. A mixture of sulfuric acid with chlorous acid, chloric acid, or potassium chlorate also produced a red color; this color was not stable, it disappeared after a few

minutes. A violet color was produced by a mixture of sulfuric acid with lead dioxide, chromic acid, chromates, manganese sulfate, iodic acid, and most iodates (Lefort, 1844b).

Morphine

In 1851 Lefort published a long memoir describing the chemical and toxicological effects of morphine during its passage through the animal economy (Lefort, 1861). He wrote that the action of vegetables alkalis on the animal economy and its medical-legal aspects were of special interest. In fact, some of these substances, especially those among the narcotic and narcotic-acrid poisons, had, in their mode of action, so strong similarities that the morbid symptoms left in the mind a doubt, which was still increased by the difficulties of isolating with certainty these poisons from the materials that accompanied them. Morphine was the extreme example of this problem because it was credited with most of the active and poisonous properties of opium, and in addition, it was the most frequent cause of poisoning (Lefort, 1861).

According to Lefort, most of the experiments performed in France and Belgium proved that clearly defined vegetable alkalis such as morphine resisted without alteration the most active and extended putrefaction of animal matter, such as the one taking place during corpse decomposition. This was a very important property in legal medicine because it allowed the successful analysis of this poison, even when present in very small amounts. Two tools were available for achieving this purpose. The first, the most reliable and also the most delicate and difficult, consisted in obtaining morphine in the purest state, in order to determine its crystalline form, its solubility in different solvents, its physical and chemical properties, and its chemical reactions. The second tool was based on the specific coloration communicated by certain reagents to a concentrated solution of the alkali, for example, red with nitric and iodic acids, and blue by salts of iron sesquioxide (Lefort, 1861).

Lefort emphasized that the most important goal of the analysis was the maximum, if not total, separation of the morphine contained in the vomiting, stomach, food, or suspected liquids. Morphine was so soluble in concentrated alcohol that its separation was very easy, as long as the material analyzed did not contain other substances equally soluble in alcohol, particularly if they colored the resulting solution. Regarding the bleaching of the alcoholic solution, Lefort shared the opinion of legal physicians who rejected using animal charcoal for this purpose because the carbon strongly retained part of the poison. He had found that mixing an alcoholic solution containing 0.01 g of morphine with 100 g of animal charcoal perfectly washed, resulted in the partial recovery of the alkaloid, even after five successive washes with cold or hot alcohol. Lefort disagreed with the finding of Jean Servais Stas (1813-1891) that alkaloids such as morphine, codeine, and strychnine could be easily separated with ether from their liquid solutions (Stas, 1851, 1854; Lefort, 1861). Lefort was surprised that Stas had included morphine in his results when it was a known fact that this alkaloid was almost insoluble in ether. Nevertheless, he carried on the following experiment: He dissolved 0.1 g of morphine acetate in water acidulated with tartaric acid (1 g of acid/100 g of water) and then supersaturated the solution with KOH. He added all to a flask containing 60 g of ether and left the whole alone, under agitation, for several days. The liquid was filtrated and then left in air to evaporate alone. The solid residue was found to contain an extremely small amount of morphine. Analysis of the initial alkaline solution indicated that it contained practically all the raw morphine. This result confirmed that Stas' method was inappropriate for morphine (Lefort, 1861).

Some chemists believed that the presence of morphine could be assured only after it had been separated in a very pure state from the suspected sample. Only in this form it could be tested for his color, taste, and crystalline form. Lefort wrote that the problem was not that simple. Usually, a very small amount of morphine was accompanied by a large amount of organic material (vomits, stomach, intestines, etc.) containing all kinds of colored substances and solutes; most of these able to dissolve the morphine. All this made the analysis extremely difficult and of low accuracy. He did not explain in detail the appropriate procedure, only indicated the any chemical agent that precipitated completely the albuminus matter, fats, etc., was appropriate. It was up to the expert to decide if he used lead acetate, silver nitrate, or tannin. Anyhow, it seemed that the best reagent for this purpose was silver nitrate because it precipitated best the animal matter and bleached the suspected solutions almost completely. As mentioned above, the best coloring reagents were nitric and iodic acids and the salts of iron sesquioxide. For this reason, Lefort decided to study in detail the sensitibity of these two compounds (Lefort, 1861).

It was a known fact that treating crystalline morphine, powdered or in concentrated solution, with nitric acid produced a blood red coloration. Nevertheless, it was also known that other organic alkalis (i.e., impure brucine and strychnine) produced the same reaction, particularly when in the presence of sulfuric acid. Hence, this reaction was a necessary but not sufficient test for the presence of morphine. In 1839 Georges Simon Sérullas (1774-1832) had shown that mixing a solution of morphine with iodic acid or an acid iodate resulted in a red coloration and deposition of iodine (Sérullas, 1830). In 1836 Pierre-Joseph Pelletier (1788-1842) discovered that while strychnine, brucine, cinchonine, quinine, and codeine reacted with iodic acid forming definite salts, morphine behaved in a completely different manner. Thus, mixing a solution containing morphine with another of iodic acid resulted,

initially, in rose coloration of the liquid and precipitation of iodine. Afterwards, the rose substance reacted as an alkali with the free iodine and formed an orange-brown mass containing iodoform. Withdrawing the free iodine with starch produced a slightly colored liquid, which became more intense upon addition of aqueous ammonia (Pelletier, 1836). Experiments conducted by Lefort showed that his reaction was extremely sensitive and indicated the presence of morphine on solutions containing 1/10,000 part. This solution was tinted pale yellow by iodic acid but addition of a few drops of ammonia intensified the color significantly. Lefort used the following procedure to obtain solid morphine: the solution of morphine was put in a porcelain capsule having a flat basis, and then strips of very white paper were introduced in the liquid and afterwards dried over a water bath. This operation was repeated several times, producing a substantial absorption of the liquid in the paper. The end result was dry morphine deposited on the paper. The presence of morphine was easily shown by the reaction with nitric acid, iodic acid, or salts of iron sesquioxide. Lefort found that the use of glue-free paper allowed keeping the sample unaltered for a long time (Lefort, 1861).

In 1815 Stéphane Robinet (1799-1869) discovered that mixing a concentrated solution of ferric chloride with another of morphine resulted in the instantaneous formation of a strong blue liquid, which became deeper upon addition of water (Robinet, 1825). Additional experiments by Lefort showed that in the presence of an excess of salt the blue coloration turned green by the combination of the yellow hue of the salt with the blue color of iron morphite. In addition, a 1% weight solution of morphine in water still produced a blue solution; further dilution to 1/300 parts of morphine resulted in a pale-yellow liquid. For these reasons, it was recommended that the tested solution of morphine be as concentrated as possible. This was easily achieved using the technique with glue-free paper described above (Lefort, 1861).

It was usually accepted that the morphine (or is salts) introduced in the animal economy went unchanged through the circulatory system, was particularly felt by the nervous system, and eventually eliminated by the renal system. Nevertheless, the experimental evidence on the presence of morphine in the urine or the blood was conflicting, some researchers had found i, others, had not. Lefort tried different procedures to try to clarify this problem. It was known that putrefaction was hindered by substances such as mercuric chloride, silver nitrate, mercuric oxide, essential oils, SO_2, and wood vinegar, and it also was accepted that besides distilled water, all ordinary solvents such as alcohol ether and chloroform, reacted with urea in the manner as morphine. This meant that this substance was the principle that did not allow the neat separation of small amounts of morphine dissolved in a large volume of urine, particularly the one discharged during the night (known to be richer in urea). Lefort found that morphine dioxide nitrate, slightly alkaline, was able to precipitate the urea from its solutions. The only problem was that in the presence of alkalis, morphine reduced the salts of mercuric dioxide and that the filtrated solution always contained traces of the mercuric salt. This resulted in the total destruction of the alkaloid during the concentration of the solution, and precipitation of metallic mercury (Lefort, 1861).

Lefort also investigated the possibility that morphine was eliminated by transpiration. Substances such as potassium iodide and the acids benzoic, cinnamic, and tartaric, were found in the sweat of individuals that used them as medicine, while others, such as quinine, lactose, and salicin, were not. Lefort macerated in alcohol cloth articles of a morphine consumer that in summer were known to have been strongly in contact with sweating parts of the body. The resulting yellow alcoholic solution was concentrated by evaporation over a sand bath, until reduced to a small volume. This residue showed no reaction with nitric acid and with ferric chloride, the classical reagents for morphine. Reaction with iodic acid yielded a liquid having a rose coloration that disappeared with ammonia, indicating the presence of morphine or of urea. Further analysis indicated that the active component with urea nitrate. All the above results indicated that morphine, administered in continuous small doses, was able to travel along all the animal economy and be eliminated by the urine but not by sweat (Lefort, 1861).

Lefort reached the following conclusions: (1) under no circumstances charcoal should be used for bleaching solutions suspected of containing morphine; (2) Sas's purification method should not be applied to morphine; (3) the colored reaction of nitric acid and morphine had analytical values only if supported by other results; (4) the salts of iron sesquioxide were the best reagents for assuring the presence of morphine, as long as the alkaloid was in powder form or as a concetrated solution; (5) iodic acid was not a definite reagent for signaling the presence of morphin, except if it was mixed with ammonia. In this case the coloration produced only was typical to morphine; (6) iodic acid mixed with ammonia could detect morphine in a liquid containing only 1/10,000 part of the alkaloid; (7) using paper free of glue allowe obtaining solid morphine distributed over a large surface, allowing bringing to light the reactions that it experimented with different identfying reagents, and (8) morphine ingested in a continuous manner, in variable doses, would be present in the urine but not in the sweat (Lefort, 1861).

Digitoxin (digitaline)

In 1824 Auguste Le Royer reported the separation of the active principle of foxglove (*Digitalis pupurea*) (Le Royer, 1824). He extracted one pound of the commercial plant first with cold ether and then with hot ether in an autoclave. The resulting yellow green bitter extract was concentrated by evaporation to a resinous residue of

unbearable bitterness, which gave to the tongue the same feeling of numbness felt by chewing aconites (wolfsbane). Addition of water dissolved the residue partially and left a deposit of impure chlorofill, contaning part of the bitter principle, which could not be eliminaed even after numerous hot washes. The acid liquid phase was neuralized with lead hydroxide, which separated the acid and also the active principle. The filtrate was evaporated to dryness and extracted with ether; elimination of the solvent left a brown sticky substance, of basic character and deliquescent. Le Royer verified that it was the active principle by injecting in the abdomen of a rabbit one g of the principle dissolved in 177 g of distilled water. The animal died in a short time, without anguish. The same effect was observed after injecting in the veins of a cat a solution containing one gram of the principle dissolved in 354 g of distilled water. The cat died in 15 minutes without major suffering (Le Royer, 1824).

In 1844 the Sociéte de Pharmacie de Paris offered a prize to the scientist that would separate the active principle in a pure form. This prize was earned by Homolle (Homolle, 1845). He macerated with water one kilo of the dried leaves of *Digitalis,* coarsely powdered and previously moistened. The extract was immediately precipitated with a slight excess of lead sub-acetate. The filtrate was limpid and almost colorless, preserving the original bitterness and presenting a slight acidity. After neutralization with sodium carbonate, filtration, and further purification, the resulting alkaline brown liquid was precipitated with tannin. The new precipitate was a soft paste that was dried, powdered and extracted with concentrated alcohol. Evaporation of the alcoholic extract left a granulated mass containing the bitter principle (which Homolle named *digitaline*) mixed with part of the impurities. Digitaline was described as a white and colorless substance, difficult to crystallize, and solidifying most frequently as porous warty masses of small laminae. It was so intensely bitter that 4 mg were enough to communicate a strong bitterness to one liter of water. Elemental analysis indicated that digitaline did not contain nitrogen. Homolle added that digitaline had a characteristic property that differentiated it from all other known substances: it formed a beautiful emerald-green solution with concentrated HCl. A small particle of it, placed in a tube with 2 or 3 drops of HCl, was sufficient to develop promptly the green color. This property could well be considered the proper criterion for discovering digitaline in medico-legal or analytical research. Homolle also reported the physiological effects of digitaline in humans and different animals: 0.01 g applied under the skin was sufficient to produce all the poisonous effects, as headache, dimness of sight, general debility, shivering, diminished urinary secretion, irregularity, and intermittence of the pulse without alteration in its frequency (Homolle, 1845).

Lefort speculated that perhaps, for toxicological studies, it would be possible to separate the digitaline by the dialysis process developed by Thomas Graham (1805-1869). He found that commercial digitalin was marketed in two forms, a secret one, developed by the Merck Company, and a second one, produced by the Homolle process. This forced him to determine the physical and chemical properties of both varieties. The Merck product was yellow white, neutral to litmus paper, totally soluble in water and alcohol, and little in ether, carbon disulfide, and benzene. Tannin deposited it completely from its water solution. Added as powder to concentrated HCl, it dissolved immediately giving a solution initially yellow, afterwards brown, and finally green. At this stage, the solution became turbid and deposited a brown substance, which seemed to be a combination of digitaline, or one of its principles, with HCl. Contacting digitaline with HCl vapors turned it yellow and then brown, while changing to a pasty state. Microscopic examination indicated that the Merck digitalin was composed of small and very translucent irregular fragments (Lefort, 1864).

The French digitaline was yellow white, little soluble in cold water, ether, carbon disulfide, and benzene, and very soluble in alcohol. It was also precipitated by tannin. Mixed with HCl it turned first yellow and then pale green and dark green. When in the green state it became turbid and deposited a dark green substance. Its behavior with HCl gaseous was very different from that of the Merck variety: it assumed first a yellow color, then brown, and finally deep green, while turning into a semi-liquid state and releasing a strong smell of powdered digitalis leaves. Lefort remarked that this last characteristic was one of the best procedures for detecting the presence of digitaline in colorless liquids. Microscopic examination indicated that the French digitaline appeared as an opaque magma, having a granular and utricular aspect. A translucent substance separated the small utricular masses. This result indicated that the insoluble digitaline was composed of more than one substance. It seemed that the insoluble digitaline contained, apart from other principles, a volatile substance that gave it its typical odor and had the property of turning green under the action of HCl (Lefort, 1864).

According to Lefort, Graham had shown that organic substances having regular crystalline forms, crossed well a parchment barrier, and also, that agents such as HCl activated the diffusion of less crystallizable substances. Lefort experiments demonstrated that an alcoholic solution of the insoluble and soluble forms of digitaline crossed the parchment without difficulty, leaving behind the other components that accompanied it, naturally or accidentally. The natural bitterness of both varieties of digitaline, and the particular smell they released with HCl gas (and the pertinent precipitate), constituted sufficient characteristics for confirming the presence of digitaline in a forensic examination (Lefort, 1864).

In a following paper Lefort added additional information about the differences between the two varieties of digitalin he had examined (Lefort, 1867b). On the one hand, the preparation of the Merck product was still a secret but seemed to be based on the seeds of the plant; on the other hand, it was well known that the insoluble variety was prepared from the leaves of the plant. In France the seeds were very expensive because they were not used for pharmacological purposes. In Germany they were the purpose of a large crop because they were completely used to fabricate digitaline. It was now known that the leaves of digitalis contained both varieties of digitaline, with an abundance of the insoluble variety. In the seeds the relation was the opposite, they contained a larger percentage of the soluble variety. It was possible that the soluble and insoluble varieties had a slightly different chemical composition, or that they were two isomers produced at different maturity stages of the plant. It was known that the degree of maturity of the plant influenced the quantity and the quality of each class; for example, the digitaline obtained after flowering was more soluble than the one produced in the previous stage. If true, these facts indicated that the soluble digitaline was a derivative of the insoluble one. These results provided a possible explanation about the conflicting information regarding the physiological effects of digitaline. The soluble variety would probably be more disruptive of the animal economy because it would be totally absorbed after being ingested (Lefort, 1867b).

Emetine

The beginning of the 19th century was characterized by the discovery of many new active principles contained in vegetable sources brought to Europe from the New World. Among these was the isolation of emetine, the active principle presents in ipecacuanha, by François Magendie (1783-1855) and Pierre-Joseph Pelletier (1788-1841) (Magendie & Pelletier 1817). Little chemical information was available about ipecacuanha, it was not known if its emetic powers were due to a particular substance, which could be isolated, and if it was the same in the different species of the plant, brown (*Psychotria emetica*), grey (*Calicocca-ipecacuanha*), and white (*Viola emetica*) (Magendie & Pelletier 1817). Magendie and Pelletier succeeded in isolating the active principle present in the cortical part the root of the brown species as a bitter, almost odorless yellow-red extract, soluble in cold water and having very strong emetic powers. Further analysis indicated that the root contained, by weight, 2% of fatty material, 16% emetic substance, 6% wax, 10% gum, 42% starch, and 20% woody material (the remaining 4% were analytical losses). In addition, Magendie and Pelletier believed that the root also contained an acid, most probably gallic acid. The emetic substance crystallized as red brown scales; it was nauseating and had a bitter taste. Upon distillation it yielded water, acetic acid, CO2, oil, and left a carbonaceous porous residue. Further analysis indicated that it did not contain nitrogen. The same emetic substance was found to be present in the three species of ipecacuanha studied, for this reason it was named *emetine*, from the Greek ἔμετος (*émetos* = *vomiting*). Toxicological studies on cats and dogs showed that the administration of a few milligrams resulted in vomiting, drowsiness, and return to normal after a long period of time. The autopsy revealed that the animals had developed a strong inflammation of the lung tissues and of the mucous intestinal membrane (Magendie & Pelletier, 1817). In 1822 Dumas and Pelletier reported an improved method for preparing emetine, this time the product was white and sometimes yellowish, pulverulent, stable in contact with air, melting at about 50 °C, little soluble in water and more in hot water, very soluble in alcohol, and practically insoluble in ether and oils. It had a basic character and reacted with acids without producing crystallizable salts. Elemental analysis indicated that it contained, by weight, 64.57% carbon, 7.77% hydrogen, 22.95% oxygen, and 4.30% nitrogen (0.41% losses), corresponding to the formula $C_{30}H_{22}NO_3$ (Dumas & Pelletier, 1823).

In 1851 Willick demonstrated that the acid present in ipecacuanha was not gallic acid, as assumed by Magendie and Pelletier, but a new acid that he named *ipecacuanhic acid* (Willick, 1851). This acid appeared as a brown-red substance, very bitter and hygroscopic, soluble in ether, alcohol, and water. Its aqueous solution did not react with neutral lead acetate but with the triacetate it produced a white-brown precipitate. Elemental analysis indicated that it contained, by weight, 56.37% carbon, 6.01% hydrogen, and 37.59% oxygen, corresponding to the formula $C_{15}H_8O_6$. According to Willick, this acid was analogous to the coffeo-tannic acid present in diverse plants of the Rubiaceae, and, particularly, in the grains of Arabic coffee (Willick, 1851).

In his first paper on the subject, Lefort wrote that a new variety of ipecacuanha had been discovered in New Granada that had properties somewhat different from the one from Brasil. The roots of this plant had not been quite accepted by the pharmacists because it was claimed it contained emetine mixed with other active components. This argument led Lefort to make a comparative study between the two varieties (Lefort, 1869a). He mentioned that Dumas and Pelletier had reported that the reaction between emetine and tannin produced a precipitate highly insoluble in water. Lefort decided to use this reaction to determine the amount of emetine present in each variety of ipecacuanha. Known weights of dry powdered roots were extracted first with concentrated warm alcohol and then with alcohol of 50% volume. The combined extracts were evaporated in a water bath to a syrupy consistency and the residue diluted with 15 to 20 times its volume of distilled water. The filtrate was treated with a concentrated solution of tannin and the precipitated emetine tannate separated, washed, dried, and weighed. This procedure had the advantage that this tannate was not decomposed by alkaline and

terreous oxides, and hardly by lead oxide or hydroxide. The results indicated that the ipecacuanha of New Granada contained about 23% less emetine than the one from Brasil. Lefort found that emetine nitrate was sparingly soluble in water. Thus, at 50 °C, an aqueous solution of emetine acetate mixed with potassium nitrate, gave a voluminous precipitate, insoluble in water and very soluble in alcohol. Lefort used this reaction to dose emetine by an alternative procedure. He found that the amounts of emetine nitrate precipitated from 100 g of ipecacuanha from Brasil and New Granada were 1.350 and 1.082 g, respectively. These results indicated that the ipecacuanha of Brasil was more valuable than the one from New Granada; the latter should be used in times of scarcity of the former (Lefort, 1869a).

Lefort, based on the available information, developed the following procedure for extracting emetine (Lefort, 1869bc): Powdered ipecacuanha was exhausted by displacement first with alcohol of 86% volume and then with alcohol of 56% volume; the combined extracts were evaporated in a water bath to syrup consistency. This residue contained the emetine combined with the ipecacuanhic acid reported by Willick. The residue was then treated with a concentrated KOH solution and chloroform. The chloroform phase, containing the separated emetine, was distilled, leaving a brown residue containing the emetine and the resinous material that Magendie and Pelletier had reported as non-vomiting. Treatment with a weak acid eliminated the impurity and treatment with aqueous ammonia separated the emetine. The purified emetine appeared as a white or slightly grey powder, bitter and almost odorless, melting at 70 °C, soluble in concentrated alcohol and chloroform, sparingly soluble in ether and fatty oils, and non-crystallizable. Lefort described the reaction of emetine with a variety of reagents, among them, KOH, NaOH, ammonia, HCl, sulfuric, phosphoric, and acetic acids, nitric acid, tannin, etc. Lefort prepared emetine sulfate and hydrochloride and from their composition deduced that the formula of emetine was $C_{60}H_{44}N_3O_{16}$ (Lefort, 1869bc).

A further paper by Lefort and Frédéric Würtz described an improved procedure for preparing pure emetine based in the previous discovery that emetine nitrate was sparingly soluble in water (Lefort & Würtz, 1877). Lefort and Würtz used this reaction to neatly separate emetine from all its foreign impurities. The nitrate was prepared by reacting a concentrated extract of ipecacuanha with a concentrated solution of potassium or sodium nitrate. The precipitate was washed several times with water, then dissolved in alcohol and treated with limewater. The mixture was evaporated to dryness over a water-bath and the powdered residue treated with ether. The ethereal extract was purified with sulfuric acid and ammonia. The final product, pure emetine, appeared crystals shaped as long needles, having a composition corresponding to the formula $C_{22}H_{20}NO_3$ (Lefort & Würtz, 1877).

Phosphorus and putrefaction

Lefort wrote that he was interested in the study of the fermentation phenomena that accompanied putrefaction, without inquiring on the nature of the ferment that produced it, and without touching the question of spontaneous generation (Lefort, 1874a). Chemists agreed that phosphates were always part of beer yeast. Pasteur had gone further and admitted that phosphates were the true food of ferments; without its presence cellules could not form and fermentation could not be established. Lefort mentioned that in 1866, Claude Collas, a Parisian pharmacist, had reported that an aqueous solution of fish glue, containing in suspension a small amount of gelatinous calcium phosphate, putrefied in a smaller time that the same glue without the salt. In addition, fresh beef meat minced with a small amount of calcium phosphate, had begun putrefying after 30 hours while another meat portion, non-salted, did not undergo putrefaction for seven days. Collas wrote that calcium phosphate was obviously not ferment but as claimed by Pasteur, "it contained the elements necessary for the development of the spores, suspended in the air, of the special ferment" (Collas, 1866). Lefort added that terreous salts such as the bicarbonates of calcium and magnesium, magnesium sulfate, and the nitrates of magnesium and calcium, did not accelerate the putrefaction of muscle flesh, actually they retarded it a little. If this theory was true, normal secretions of the organism, loaded with phosphates like urine, should not ferment differently from the same secretions mixed with calcium phosphate. He tested this idea and found it to be correct. Samples of both urines left alone in the air turned alkaline in about the same length of time. In addition, if calcium phosphate was the main agent of the multiplication of the animal ferment, then, fleshy masses that contained the most of this salt should decompose faster than those containing less. It was known that fish muscles decomposed faster than beef meat. An easy test was comparing the weight of cinder produced by these foods. Lefort's results indicated that 100 parts of cinders of perch and carp contained 44.34 and 42.20 parts of terreous phosphates, respectively, while the same weight of beef and veal meat contained only 20.60 and 26.40 parts of the same components. Many additional experiments with meat of different origins proved this statement to be true. Purulent infections were most readily produced in affections of the marrow of bones; this was because the central part of the bone, being less dense and more attackable than the periosteum, yielded calcium phosphate more readily (Lefort, 1874a).

It was known that putrefying bodies released an odor similar to that of garlic and that putrid fermentation was accompanied by a slight emission of light. It was assumed that a sulfur derivative caused the garlic odor, and the phosphorescence, a combination of hydrogen and phosphorus. Lefort conducted a series of experiments to test

these assumptions and found that the source of the odor and light was not phosphine but a phosphorus sulfide that was formed as follows: As soon as the elements of the fibrin and of the protagon became separated and those of the sulfur and phosphorus present in the tissues combined, they generated the odor of putrid substances. Since phosphorus sulfide was very unstable, it decomposed giving-forth light as soon as it encountered water and air, and hydrogen sulfide and phosphoric acid. This explained also why the phosphorescence phenomenon was so feeble and short lived. Lefort added that without putting phosphorus sulfide in the same rank as septin (a soluble poison in the blood), it is reasonable to assume that the sulfide, on account of its toxic properties, was the cause of certain accidents originating from the ingestion of certain preserved foods, such as such as salt fish, pork, butchers' preparations, and particularly pudding, sausage, Italian cheese, etc. Phosphorus once oxidized to phosphoric acid or phosphates, could not be reduced by fermentation, and combined with sulfur, but it influenced the fermentation by facilitating the multiplication of the germs of the ferment. For this reason, waters containing phosphates should not be used for the cleansing of wounds, but distilled water, or rainwater should be preferred (Lefort, 1874a).

Preparation of atropine from belladonna leaves (*Atropa belladonna*)

Lefort carried a detail study of the relative amount of atropine present in the leaves and roots of belladonna during its different stages of development (Lefort, 1872). The leaves were composed of cellulose, chlorophyll, an atropine salt, a foul-smelling principle, and the fatty or waxy substance proper of all vegetables. The belladonna was isolated using the fact that the double iodide of potassium and mercury was the best precipitant of vegetable alkalis from their aqueous solutions containing also colorant principles and other impurities. For this purpose, Lefort employed a solution containing 9.00 g of mercury chloride and 32.50 g of potassium iodide, per 100 g of water. One hundred grams of finely powdered leaves or roots were totally dried in a stove and then extracted four times with warm alcohol of 80º. The combined extracts were filtered and distilled to eliminate most of the alcohol. The residue was mixed with a small of water and then treated again with and slight excess of the aqueous double iodide until no more precipitate was formed. The precipitate was filtered, washed with water, and dried in a platinum capsule. The hard and transparent residue of the compound of atropine with the double iodide was found to be insoluble in water, very soluble in alcohol, and stable in contact with air. The results indicated that the leaves collected during May (before flowering) contained about 15% less atropine than those collected in August (0.409 g against 0.462 g per 100 g of dry leaves). These same results were approximately obtained for leaves from wild belladonna (Lefort, 1872).

Additional results also indicated that the dry leaves contained 3% of a fatty material colored green by chlorophyll and that they scattered the foul-smelling odor proper of Solanaceae; the roots contained 1% of the same fatty matter, colored yellow and spreading a less pronounced bad odor. It was known that each year, the roots added another rootlet; their analysis indicated that each rootlet had a different content of atropine. Lefort found that the root system of roots formed during the first three years and during the first 7 to 8 years, contained about 0.4802 g and 0.2820 g of atropine per 100 parts of dry roots, respectively (Lefort, 1872).

The results indicated that the leaves of the plant contained 4.5 g of the alkaloid to the kilo and the root 2 to 5 g, depending on the age of the plant. Lefort proposed the following process for producing atropine in large quantities: the dried leaves of belladonna, slightly broken, were cleaned of all its soluble parts with boiling water containing 10 g of tartaric acid per kilo of leaves. The addition of the acid facilitated the dissolution of the atropine enclosed in the vegetable cells. The extract was evaporated to a soft mass, producing a residue of about 200 g/kg. Mixing the residue with one liter of concentrated alcohol and heating to about 50 ºC, dissolved all the atropine tartrate and distillation of the extract recovered most of the alcohol employed. The remaining material (about 50 g) was treated with ether to eliminate the resin and chlorophyll entrained, while the atropine tartrate, insoluble in ether, remained in the paste. The atropine tartrate was split by means of a concentrated solution of KOH and the free atropine purified by washes with ether, water acidulated with sulfuric acid, sodium bicarbonate, and distillation (Lefort, 1872).

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