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Mathieu Joseph Bonaventure Orfila
Contribution to toxicology

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ABSTRACT

Mathieu Joseph Bonaventure Orfila (1787–1853) was a Spanish physician and scientist, naturalized French, considered by many to be the father of toxicology. He carried basic and practical studies on many theoretical and experimental aspects of physiology, toxicology, and forensic science, and developed appropriate detection methods for small quantities of the most common poisons (mercuric chloride, arsenic, phosphorus, morphine, and hydrogen cyanide), including the particular situation of corpses buried short or long times.

Keywords: arsenic, corpses, poisoning, poisons, toxicology.

RESUMEN

Mathieu Joseph Bonaventure Orfila (1787–1853) fue un médico y científico español, naturalizado francés, considerado como el padre de la toxicología. Llevó a cabo estudios prácticos sobre una variedad de aspectos teóricos y prácticos de fisiología, toxicología y ciencia forense. Desarrolló métodos analíticos de detección adecuados para cantidades pequeñas de los venenos más comunes (cloruro mercúrico, arsénico, fósforo, morfina, y ácido cianhídrico), incluyendo la situación particular de cadáveres enterrados por períodos cortos o largos.

Palabras clave: arsénico, cadáveres, envenenamiento, venenos, toxicología.

INTRODUCTION

Life and career (Anonymous, 1853; Bérard, 1855; Fayol, 1930; Hernandez Mora, 1853; Dubois, 1854; Bertomeu-Sánchez & Nieto-Galan, 2006)

Many publications are available describing different aspects of the life and career of Mathieu Joseph Bonaventure Orfila (1787–1853) (A large bibliography about Orfila and its work can be found in http://www.bium.univ-paris5.fr/histmed/medica/orfila.htm). Here we resume the most significant aspects.

Mathieu Joseph Bonaventure Orfila (Figure 1), a Spanish naturalized French physician and scientist, born in Mahon (Minorca) on April 14, 1787, was the son of a prosperous maritime merchant.

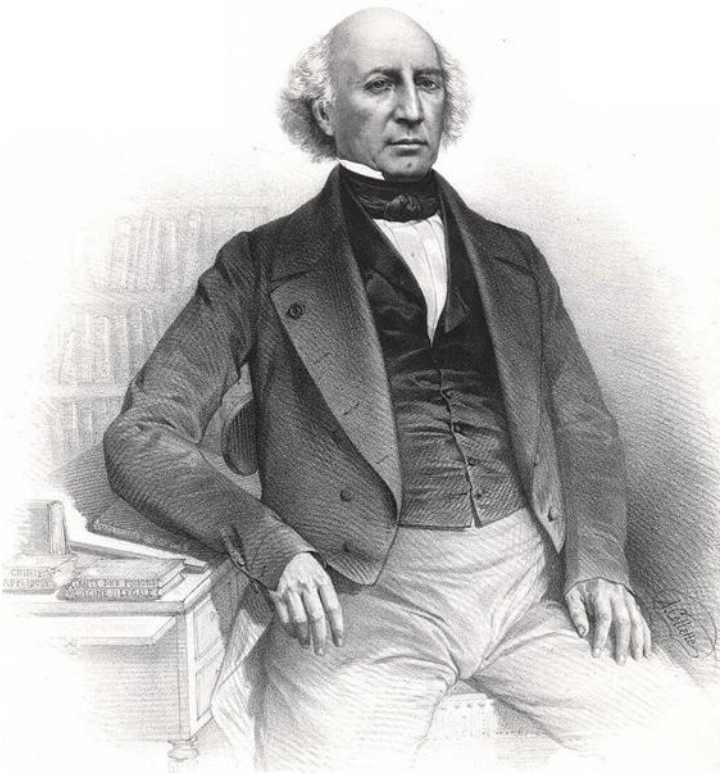

Fig. 1. *Mathieu Joseph Bonaventure Orfila*.

In 1804, after receiving a solid basic education at home, he enrolled in the Faculty of Medicine of Valencia and then transferred to the University of Barcelona where he followed the chemistry course given by Francesc Carbonell (1768-1837) at the School of Chemistry, His successful studies led the city government to award him a four-year scholarship to study two years with Joseph-Louis Proust (1754-1826) (which unfortunately had already abandoned Spain) in Madrid, and two more with Antoine-François Fourcroy (1755-1829) in Paris. While in Paris he worked with Fourcroy and Nicolas Vauquelin (1763-1829) while following doctoral studies at the Faculty of Medicine of Paris. In 1811 he was awarded the degree of docteur ès-médicine after successfully defending a thesis about the chemical characteristics of the urine of persons affected by jaundice (Orfila, 1811). In the following years he maintained himself by giving private lessons in chemistry, forensic medicine, and anatomy. In 1816, he became royal physician to the French monarch Charles X (1756-1840) and in 1817 he succeeded Louis-Jacques Thenard (1777-1857) as chemistry professor at the Athénée of Paris. In 1819 he won by competition the chair of Legal Medicine at the Faculty of Medicine of Paris, an appointment that forced him to become a French citizen. In 1822 he replaced Vauquelin at the chair of Medical chemistry and in 1830 he was elected dean of the Faculty of Medicine, a position he kept until 1848. The Revolution of 1830 dethroned Charles X and replaced him by Louis Philippe (1773-1850). Orfila maintained his position of royal physician and began a prosperous academic and professional career that terminated abruptly in 1848 with the popular uprising that forced the abdication of Louis Philippe. Orfila's support of the monarchy led to his arrest, loss of all his appointments

(except his professorship), and his deanship. In 1851 he was rehabilitated and elected President of the Académie de Médecine.

Orfila passed away on March 12, 1853, after suffering from pneumonia, and was buried in the Montparnasse cemetery (Figure 2).

***Fig. 2**. Tomb of Mathieu Joseph Bonaventure Orfila, Montparnasse Cemetery, Paris*

In his will Orfila left a significant sum of money in favor of numerous scientific and charity activities, among them the establishment of a prize on medical-legal medicine, the Faculty of Medicine of Paris and the School of Pharmacy, the preservation of the Museum of Comparative Anatomy, now called the Musée Orfila, and the medical benevolent society.

Orfila participated actively in scientific and public activities and was recognized accordingly. He was member of many scientific societies, for example, of the Académie Nationale de Médicine, the Société Médicale d'Émulation, the Société de Chimie Médicale, Conseil Général des Hospices, Conseil Supérieure de Instruction Publique, Real Academia de Medicina y Ciencias Naturales of Madrid, Seville, Cadiz, Barcelona, Santiago, Murcia, Baleares Islands, of Berlin, Belgium, and Livorno; corresponding member of the Institute (former and afterwards the Académie des Sciences). He also served as President of the Association de Prévoyances des Médecins of Paris, etc. In 1821 he was appointed Officier of the Legion d'Honneur and promoted to Officier in 1833 and to Commandeur in 1838; he was also appointed to the Real y Distinguida Orden Española de Carlos III, to the Order of Saint-Anne of Russia, officer of the Order of Leopold of Belgium, and to the Ordem Nacional do Cruzeiro do Sul of Brazil. He was also member of the Universities of Dublin, Philadelphia, and Hanau.

Orfila was the founding editor of the medical journals Journal de Chimie Médicale, de Pharmacie et de Toxicology in 1824 and the Annales d'Hygiène Publique et de Médecine Légale in 1829.

Scientific contribution

Orfila wrote about 50 papers and books (e.g. Orfila, 1815, 1817b, 1821-1823, 1824, 1825b, 1831, 1841, 1843b) on the subjects of physiology, toxicology, forensic science, etc. In addition to the few subjects described below, he also studied the poisoning by barium chloride (Orfila, 1818b), cantharides (Orfila, 1818c), bleach, delphin, and gallnut (Orfila, 1821), salts of lead, bismuth, tin, silver, gold, and zinc (Orfila, 1842b), nicotine and conicine (Orfila, 1851); etc. In addition, he reported the discovery of a biliary stone having no adipowax and an excess of the yellow matter (Orfila, 1812); he studied the blue coloration of albumen under the action of HCl (Orfila, 1829c); the possibility of using the color of the hair for

identification purposes (Orfila, 1835), and of the phenomenon of suspension (of biological activity before death; no signs of life) (Orfila, 1840b); compared cerebral matter with other body organs (Orfila, 1850); etc.

Poisonous metals

Orfila wrote that a particularly difficult situation in forensic science was the search of poisonous metals present in colored liquids such as red wine, coffee drink, etc. because the pertinent reagents produced colored precipitates or solutions, and others, such as chlorine, had the property of bleaching the solution (Orfila, 1820). Orfila provided the analytic procedure to follow in the case of white arsenic oxide, arsenic acid, potassium acid arsenate, mercuric chloride, cupric acetate, potassium antimony acid tartrate (emetic tartar), lead compounds, bismuth acid nitrate, zinc sulfate, gold chloride, stannous chloride, stannic chloride, barium chloride, and a mixture of indigo and sulfuric acid (Orfila, 1820).

Opium and morphine

The first work of Orfila on morphine was devoted to the action of this alkaloid on the animal economy with the purposes of proving that the water extract of opium owed its medicinal properties to the alkali containing hydrogen, carbon, and nitrogen discovered by Friedrich Wilhelm Sertürner (1783-1841) (Sertürner, 1817; Orfila, 1817a, 1818a), and to compare the effects of this extract with those of the pure morphine extract. Pure morphine appeared as a colorless and odorless solid, heavier than water, and crystallizing as parallelepipeds. It was almost insoluble in water and easily soluble in cold alcohol and ether. The water solutions were alkaline and turned blue litmus paper. Morphine combined easily with all the acids, neutralizing them like alkalis, and yielding crystallizable salts. According to Orfila, the aqueous extract of opium acted on the animal economy only after being absorbed and transported by the blood stream. It produced the paralysis or rather the numbness of the abdominal limbs, accompanied by vertigo, tremor, complaints, convulsive motions, and death (Orfila, 1817a, 1818a). In 1803 Jean-François Derosne (1774-1855) discovered that opium contained a compound (Derosne's salt, narcotine) that he believed gave opium its particular properties (Derosne, 1803), and in 1821 Pierre-Jean Robiquet (1780-1840) discovered that in addition to meconic acid, opium contained codeine, another substance combined with morphine (Robiquet, 1821).

Orfila administered different doses of morphine, or its salts, suspended in water and an aqueous extract of opium, to small dogs having an empty stomach, in a series of 20 experiments, and reported the following results: (1) it was possible to introduce up to 0.78 g of morphine alone in the stomach of a dog without causing any sensible phenomenon, but a similar dose of an aqueous extract of opium resulted in violent poisoning, followed several times by death. The relative inaction of morphine was a result of its little solubility and the difficult of its attack by the stomach juices; (2) morphine salts soluble in water (i.e. acetate, sulfate, and hydrochloride) acted with the same intensity as the water extract of opium and produced the same symptoms, proving that the effects of this medicament had to be attributed to a morphine salt (probable the meconate) and suggesting investigating the presence of morphine in indigenous plants, its separation and conversion into salts, and substitution of the water extract by the latter; (3) it was possible to administrate large doses of the water extract of opium, clean of morphine, without causing signs of poisoning. Appearance of slight effects proved that the morphine had not been removed completely; (4) 0.39 g of morphine dissolved in olive oil acted in the same manner as 0.78 g of a water extract of opium, proving that the oil neutralized the poisonous properties of morphine, substantially less than acids, This remarkable effect allowed duplicating the medical properties of the extract; (5) morphine, as all substances that acted after being absorbed, produced a stronger action when injected in the veins, rather that applied on cellular tissue or injected in the digestive tract; (6) the poisoning effect of morphine was identical to that produced by opium and should be treated in the same manner. The first efforts were to be addressed to the elimination of the poison with emetics, followed by administration of diluted organic acids, a coffee drink, etc. These means, sometimes assisted by bleeding from the jugular vein or arm, were almost infallible; and (7) alcohol, diluted to the point that it had no effect on dogs, dissolved a very little amount of morphine, making it impossible to determine the effects of morphine. It was probably that the alcoholic solution could be used on humans, usually accustomed to alcoholic drinks,

to take stronger doses of the alcoholic solutions without experimenting the minimal discomfort (Orfila, 1817a, 1818a).

In a following paper, Orfila wrote that the role played by morphine, narcotine, and codeine in opium was still uncertain. Some thought that narcotine was an excitant while morphine was a sedative, while others believed that narcotine was inert. These inconsistencies led Orfila to conduct more trials on the subject (Orfila, 1825a). After a long series of experiments, he concluded that opium owed its poisonous properties to a morphine salt, the principle of Derosne, and a virile matter that volatilized with the water during the distillation of opium. The action of opium seemed to be the combined action of these three components, while the toxic effects were due to Derosne's salt, as shown by the fact that the extract, cleaned of this principle and still containing the morphine salt, killed the animals slowly, in about the same time interval as the ordinary extract. Derosne's salt was not the excitant of opium while morphine was a narcotic. The extract, cleaned of the principle of Derosne by ether, was as excitant as the raw extract. In addition, it was objectionable that the Derosne principle acted as a powerful stimulant when it was administered with acetic acid, because it was known that the action of this principle was stupefying or nil, depending on whether it was administered in the oil or in HCl (Orfila, 1825a).

Mercuric compounds

Orfila found that the external application of mercuric dichloride resulted in its absorption, transportation into the blood stream, and the resulting deleterious action on the heart and digestive tract (Orfila 1818c). The heart damage appeared as inflammation and perturbation of the circulation of the blood. The action the digestive tract manifested itself as inflammation of the mucous membrane near the pylorus and the rectum. These symptoms were the same as those accompanying injection in the vein or introduction into the stomach. In the latter case, death seemed to be caused by the inflammation of the contacted tissues and sympathetic lesion of the brain and nervous system (Orfila 1818c).

In forensic analysis it was easy to determine if the poisoning was due to the chloride ($HgCl_2$) dissolved in water or other solvents that did not decompose it (Orfila, 1821). It was not the same when the dichloride had been converted into monochloride (HgCl) by liquid or solid food or by the tissues of the digestive tract. This monochloride was insoluble in water and appeared combined with vegetable or animal matter that masked most of its properties. It was then necessary to focus on demonstrating the presence of metallic mercury in the suspect materials because this metal put beyond doubt the existence of a mercurial preparation. Orfila described the possible judgement error that this fact could induce (Orfila, 1821): It could well happen that an individual who had not been poisoned succumbed a few hours after swallowing 0.78 or 0.97 grams of mercury monochloride for the purpose of purging himself. The doctor required by the authority to determine the cause of death would perform the established anatomical and chemical tests and find that the tissues of the digestive canal were inflamed and that the substances which it enclosed contained metallic mercury. These results would lead him to believe that there had been poisoning, but this was not the case because the redness of the digestive canal was due to a chronic phlegmasia of which the patient was affected, and the mercury came from the small dose of calomel that he had taken, and which certainly could not have occasioned the poisoning (Orfila, 1821).

This example illustrated the importance of determining if the metallic mercury detected originated from a certain amount of mercury monochloride introduced as such in the stomach, or else, it was part of the composition of the dichloride that the individual could have swallowed and which would have been transformed into monochloride in the stomach. Orfila went on to describe in detail the procedures for distinguishing between these two cases (Orfila, 1821).

In 1822 James Smithson (1765-1829) published a short note describing a new and very sensible procedure for determining minute amounts of arsenic and mercury in solution (Smithson, 1822). According to Smithson, all the oxides and salts of mercury laid on a drop of HCl deposited on gold with a piece of tin rolled around, quickly amalgamated the gold. The resulting small voltaic cell caused the deposition of any mercury present upon the gold and turned it white. The color change took place in a few minutes or several hours, depending on the amount of mercuric chloride present. Application of heat to the ring or plate volatilized the mercury and restored the original yellow color of gold, proving the presence of mercury.

According to Orfila, the procedure was correct when a mercury salt was present but it also gave a positive answer in some cases when no mercury was present (Orfila, 1829a). For example, the tin foil

might be dissolved by the acid and then precipitated on the gold, giving the white appearance on the gold, which would disappear by the action of heat. Gold and tin, put separately into a mercurial solution, did not indicate the mercury by a white appearance on the gold. Gold, whitened by the deposition of tin and mercury, might be distinguished by pure strong HCl, which dissolved the tin and not the gold and restored the yellow color of gold. The only way to assure the presence of mercury by Smithson's method required treating first the gold plate with pure and concentrated HCl, followed by washing to eliminate the excess of acid. The clean gold was then added to the solution being tested and the whole heated in small closed glass tube. The appearance of condensed mercury was proof of the presence of the metal in the liquid (Orfila, 1829a).

In 1826 Orfila demonstrated that natural or artificial arsenic sulfides (obtained by decomposing arsenious acid by hydrogen sulfide) were poisonous as long as they were completely free of arsenious acid (Orfila, 1826). In a following paper he studied the possibility that the sulfides of lead, copper, and mercury exhibited the same characteristic (Orfila, 1829d). For this purpose, he carried several experiences in which he fed mercury sulfide (natural or synthetic) to a young dog of medium size. In the first experiment the dog was fed 0.032 g of powdered and well-washed black mercury sulfide, prepared by treating a solution of mercuric chloride with hydrogen sulfide. After seven hours the dog showed no effects, except vomiting twice, and the next day he behaved normally. In the second experiment, conducted five days later, the same dog was fed the same amount of sulfide. This time its esophagus was tied to avoid vomiting. The animal died after seven days, without presenting any other symptom than the exhaustion, which was the most usual continuation of the ligature of the esophagus. Autopsy showed no particular damage of the digestive tract, the stomach was empty and without any trace of inflammation. The large intestines were healthy and not distended. The other organs also appeared in the natural state. In the following experiments one dog was fed 0.032 g of non-washed cinnabar, and another had the same amount applied to the cellular tissue of the internal part of the thigh. During ten days the dogs showed no particular changes in behavior. In the last experiment a dog was fed 23 g of powdered vermillion and another had the same amount applied to the cellular tissue of the internal part of the thigh. Once again, the animals showed no particular changes in behavior. According to Orfila, the results of his experiments proved that the black and red mercury sulfides were innocuous (Orfila, 1829d).

In a paper published in 1842, Orfila wrote that no one had been able to prove that the absorption of mercuric chloride resulted in poisoning (Orfila, 1842bc). Many papers had discussed the absorption of mercurial preparations introduced in the stomach, or applied externally as medicines to treat venereal diseases. The purpose of the present memoir was to prove that in animals poisoned with this salt it was possible to find mercury in the liver and the urine and that the present procedures for detecting the presence of a mercurial compound in food, in the tissues of digestive tract, or in and animal viscera, were neither the surest nor the most sensitive. Orfila was also intent in providing an answer to the following questions: (1) reliability of the present tests, particularly those of Jean Louis Lassaigne (1800-1859) (Lassaigne, 1837) and Alphonse Devergie (1798-1879) (Devergie, 1836); (2) detection of mercuric chloride mixed with food liquids, as in vomits, as found in the digestive duct, or combined with some of human tissues, and (3) what happened in these various cases, did the mercuric chloride combine with the vegetal and animal substances mentioned above, or was it reduced to the state of monochloride that was sometimes precipitated alone or combined with the organic matter as an insoluble compound (Orfila, 1842bc)?

For this purpose, Orfila carried a series of twelve experiments, under different conditions, for example, three eggs were diluted in water and the filtrate, mixed with one gram of mercuric dichloride, was dissolved in distilled water. The resulting precipitate was washed repeatedly with water until the liquid was not colored by hydrogen sulfide. The clean material was then subjected to a series of treatments to determine the presence of mercuric dichloride and sodium chloride. In another experiment a similar treatment was applied to the stomach of an animal that had been poisoned by the dichloride. It was found that the sodium chloride did not contain mercuric chloride, hence, the stomach had to contain a mercurial compound because it was found to contain mercury.

According to Orfila, the work of Lassaigne proved that the precipitate formed by albumen and mercuric chloride contained, after being dried, about 5% of the dichloride. Hence it was impossible to use his method in legal medicine when it was required to detect the possible presence of a mercurial compound

insoluble in water (for example, mercury monochloride) in the state of combination in the tissues of the digestive canal, in other viscera, or in certain alimentary substances. In fact, the sodium chloride proposed by Lassaigne, did not remove this mercurial compound to the fleshy masses in which it usually existed in very small proportion (Orfila, 1842bc).

The Devergie procedure, which required dissolving the organ or other solid matter in concentrated HCl, followed by streaming the solution with gaseous HCl, should also be discarded because many times it was not enough for detecting the mercury contained in the suspected sample. Chlorine did not destroy completely the organic sample, even when being streamed through it for many hours. There always remained a yellow fatty residue resulting from the action of chlorine over organic matter. This matter was so abundant that the resulting liquids became tinted rose or brown and opposed the precipitation of mercury over a strip of copper or in the small galvanic pile (Orfila, 1842bc).

According to Orfila, a very effective procedure for detecting the presence of mercuric chloride was to carbonize the sample with concentrated sulfuric acid, in a closed vase. The resulting carbon and volatilized liquids yielded mercury and its dichloride in substantial amounts. The experimental results demonstrated clearly the absorption of mercuric chloride. Orfila had separated metallic mercury from the liver and urine of poisoned dogs, as well as from the urine of syphilis patients who had ingested small doses of a solution of mercuric chloride several days before (Orfila, 1842bc).

Hydrogen cyanide and potassium cyanide

In 1829 Orfila published a paper describing the procedures for detecting the presence and amount of HCN, dry or hydrate, present in syrup and in variety of colored and colorless liquids, the influence of the cyanhydric syrup on animal economy, and the various methods of treatment proposed to combat the effects of this poison (Orfila, 1829b). The most sensible analytic procedures were based on the odor of the sample and treating it with silver nitrate. The nose was able to identify pure HCN in amounts lower than those detectable by the most sensitive reagents. Silver nitrate reacted even with very diluted solutions of HCN producing a white curdy precipitate of silver cyanide, insoluble in water, sparingly soluble in nitric acid at room temperature, very soluble in the boiling acid, and in ammonia. The precipitate decomposed with heat and in contact with air, producing metallic silver and liberating part of the cyanogen. In addition, HCN could also be detected by cupric sulfate mixed with a little of KOH, although the resulting white precipitate could be confused with many other precipitates. Ferric sulfate, mixed with a bit of KOH, yielded a blue precipitate of Prussian blue. Silver nitrate was very useful with colorless solutions but not so with colored ones because the resulting precipitate was usually colored brown. In this case it was preferable to impregnate a strip of paper with a solution of KOH, let it dry, and then wet it with the solution being tested, followed by addition of a few drops of aqueous ferric sulfate. The paper assuming a blue green color showed the presence of cyanide. Diluting the liquid with distilled water and adding an excess of silver nitrate could be easily used to determine the amount of cyanide present. The precipitate, composed of silver nitrate pure, was separated, washed and dried. Its weight allowed a simple calculation of the cyanide present in the original solution (Orfila, 1829b).

A similar process could be used when the solution contained chlorides, carbonates, and phosphates. In this case the precipitate contained all the corresponding salts of silver. The silver phosphate and carbonate were separated by solution in cold nitric acid dissolved in distilled water; treating the residue with pure boiling nitric acid dissolved the silver cyanide and left a solid consisting of pure silver nitrate, which was handled as before. Orfila remarked that if necessary, a more accurate result could be obtained by determining the very small amount of silver cyanide dissolved in the nitric acid (he gave a detailed procedure for doing this) (Orfila, 1829b).

Orfila mentioned that the cyanhydric syrup prepared according to the French Codex contained 10% weight of HCN and had relative density 0.9. This concentration was enough to kill a robust person in 25 to 30 minutes, even when diluted with water. An interesting fact was that the intestine and stomach of dogs poisoned with HCN showed no signs of inflammation while in humans there was clearly an inflammation of the digestive mucous membrane and the small intestine. Orfila believed that the difference was caused by the fact that death in dogs took place within a few minutes after ingestion of the poison.

Orfila wrote that although no antidote was known for HCN, he had found that aspiration of water containing ammonia or chlorine, accompanied by spraying cold water over the head and the course of the

spinal cord, would be helpful. His experiments indicated that the aspiration of ammoniated water stimulated the extremely sagging nervous system; this treatment was appropriate only if applied shortly after poisoning or if the dose of the poison was high enough to kill a dog in a short period of time (Orfila, 1829b).

Orfila wrote that potassium cyanide was a chemical used in different industrial applications that could seriously affect the animal economy, depending how it had been prepared (Orfila, 1843a). Potassium cyanide lost part of its deleterious characteristics after being exposed for a long time to humid air or after being boiled in water for some time.

This salt was prepared by several methods: (1) H. A. Wigger's procedure based on streaming HCN through and alcoholic solution of pure potassium (Wiggers, 1839). The product was a white solid, having an alkaline bitter taste and a penetrating odor of HCN. It was stable at high temperatures as long as not in contact with air; it was very soluble in water and less in alcohol. Treatment with diluted acids released hydrogen sulfide, without effervescence; (2) prepared by decomposition of the yellow cyanide of potassium and iron at red-hot temperature, in a closed vessel. The resulting carbon was washed with a small quantity of water and the filtrate evaporated to dryness by gentle heat. Weak acids decomposed it with effervescence into HCN and CO_2. Treatment of an aqueous solution with limewater produced a white precipitate; and (3) prepared by calcining in a closed crucible a dry muscle flesh or blood mixed with potassium carbonate. This potassium cyanide was the cheapest one and was widely employed in industry because it dissolved the cyanides of silver, platinum, gold, etc. It was a white solid, weakly smelling like HCN, decomposed by weak acids without effervescence, releasing HCN and CO_2. The aqueous solution produced an abundant white precipitate with limewater, a white green one with ferrous sulfate, and a blue one with cupric sulfate. Heating a concentrated aqueous solution transformed the cyanide into ammonia and a solution of potassium formiate that did not affect the animal economy (Orfila, 1843a).

Orfila treated adult dogs with different doses of potassium cyanide prepared by the three methods and obtained the following results: (1) a dose of several centigrams of potassium cyanide prepared by each of the three methods killed promptly a dog and acted in the same manner as HCN; (2) The so-called potassium cyanide obtained by calcining muscle flesh or blood hardly contained potassium cyanide; it was actually composed of a mixture of potassium carbonate, chloride, etc., and was little poisonous; (3) it was true that heating a concentrated aqueous solution transformed the cyanide into ammonia and a solution of potassium formiate, but this was a very slow reaction taking much longer than 3.5 hours to occur. The same result was true when boiling the solution in the presence of air; and (4) the decomposition of potassium cyanide by the simultaneous action of the water and CO_2 contained in the air took a very long time (more than 14 hours). Potassium cyanide liquefied completely by the humidity of the air alone, kept its full poisoning properties. Hence, chemists and physicians exaggerated in their claim that dissolving potassium cyanide in water and promptly evaporating the solution to dryness resulted in the neutralization of its poisoning properties (Orfila, 1843a).

Phosphorus

Orfila and M. Rigout studied the effect of amorphous phosphorus on the animal economy, by feeding strong dogs a mixture of red phosphorus and Italian cheese (Orfila & Rigout, 1856). In the first experiment they first fed the dog 6 grams of red phosphorus, in doses of 2 grams each for three days, and on the fourth day they increased the dose to 5 grams in one portion. After seven days, no unfavorable symptoms were observed, hence they gave daily 2 grams for thirteen days, for a total of 36 grams. Since no alterations were observed, they took a more drastic action and introduced 2 grams of ordinary phosphorus into the stomach of the animal and tied the esophagus. This time the dog died the next day. Orfila and Rigout also gave to a young dog 5 centigrams of ordinary phosphorus, finely divided in olive oil. The animal scarcely lived a quarter of an hour (Orfila & Rigout, 1856)

The dog feces were analyzed after being fed both kinds of phosphorus. No particular incidents were noted with red phosphorus but those with ordinary phosphorus were found to be loaded with phosphorescent vapors.

In a third experiment, another healthy dog was fed 10 grams of red phosphorus; the dog did not eat his usual food on that day, but exhibited no signs of sickness. Two days afterwards 50 grams of amorphous phosphorus were given to the same dog: he swallowed its meat at once, but soon began to vomit. On the

same day he was again vigorous, and ate with a good appetite. After four days he took daily with his food, for four days, 13 grams of amorphous phosphorus, and then for three days 20 grams each day. After the administration of this large quantity of amorphous phosphorus, the dog enjoyed a good appetite, and the vomiting did not reappear. Some few days after taking the last dose it was killed. Not the slightest injury had occurred to the intestinal canal; the esophagus, stomach, and intestines appeared of a red color, which could only originate from the amorphous or red phosphorus. More experiments were done under different conditions (Orfila & Rigout, 1856).

Orfila and Rigout also investigated how long the phosphorus remained free in the intestines after death. Fourteen days after death, the autopsy of the dog fed ordinary phosphorus showed that it was still perfectly free from putrefaction, while another dog, not killed with phosphorus, but placed in the same position, was in a state of decomposition. In the stomach and esophagus of the poisoned dog was found a yellow, frothy substance, impregnated with the vapors of phosphorus. This substance, when heated, afforded a white flame and dense vapor, showing thereby the presence of free phosphorus. The mucous membrane of the esophagus and stomach had a strong red color. Orfila & Rigout also agitated the fluid of the stomach of the dead dog with carbon disulfide and noticed that it the separated into two layers, one watery, and the other oily, the latter consisting of the solution of phosphorus in carbon disulfide, from which the phosphorus could be isolated as a residue by spontaneous evaporation (Orfila & Rigout, 1856).

These experiences showed that 2 grams of ordinary phosphorus were enough to kill a dog and that the element could persist in the organs, in a free state, for fifteen days after the death.
It also showed that ordinary phosphorus was poisonous, but red phosphorus was not (Orfila & Rigout, 1856).

Arsenic

Orfila remarked that an important factor in the analysis of a possible poisoning by arsenic was to assure that the arsenic did not originate from the reagents, vases, metallic instruments, porcelain, and glass vessels employed in the analysis. An error of this nature could give place to terrible legal consequences (Orfila, 1839). The following items were considered; (1) sulfuric and nitric acids, potassium nitrate, alcohol, water, iron, and zinc; (2) cast iron boilers, porcelain capsules, Hesse crucibles; and (3) glass pieces such as vases, tubes, and retorts. Orfila gave a detailed description of the analytical procedure to follow for each item (Orfila, 1839).

Orfila stated that everyone agreed that when a body had been buried for a long time in a casket still undamaged, there was no chance that the surrounding ground had affected its state. Under these circumstances, no foreign body had penetrated the casket. The situation was different when the casket had cracked, had been breached, or was reduced to pieces, or when the body had been buried wrapped only in a cloth. If no arsenic was discovered in the body of someone supposed to be poisoned, could it be said that the poison had dissolved in the ground, that the poisoning that could be discovered a few days after burial would not be so after several weeks or months? If a long time after burial an expert had detected the presence of arsenic in the body, could he declare that there was no poisoning because the surrounding ground was arsenical? These questions led Orfila to study the problem in detail (Orfila, 1831,1840a).

His memoir on the subject was addressed to three questions: (1) are there arsenical cemetery grounds? (2) If the answer was positive, can these terrains provide arsenic to the surrounding corpses in such manner to induce a physician and justice to error and declare death by poisoning? And (3), could the body of an individual poisoned by arsenic release the poison totally to the soil and become free of it after a long burial? To answer the first question Orfila took a large sample of the soil from the Villey-sur-Tisle (near Dijon) cemetery and boiled it for several hours in water. The sample was taken from the section of the cemetery where a victim of arsenic poison had been buried for five months. Analysis of the filtrate in a Marsh apparatus showed no signs of arsenic. The remaining soil was diluted in water and boiled with distilled concentrated sulfuric acid. The filtrate was concentrated by evaporation and analyzed in a Marsh apparatus. This operation resulted in the deposit of very small spots of brilliant and colored arsenic. The whole process was repeated a month afterwards. This time the Marsh apparatus did not show the presence of arsenic. Several additional experiments were conducted in the same manner, for example, one with soil taken far away from the previous site; another, with soil of another cemetery were the buried person had not died of arsenic poisoning; a third one, with soil taken from a botanical garden, etc. Some of the

samples showed no sign of arsenic; others gave a positive answer. Orfila remarked that no water extract of the samples showed a positive answer (showing that no soluble arsenic compounds were present), but some of the sulfuric acid extracts did (the compound had become solubilized) (Orfila, 1831, 1840a).

To answer the second question, Orfila analyzed soils containing an arsenic compound insoluble in boiling water or soluble in cold water (for the latter case, Orfila sprayed the surface of the soil with an aqueous solution of arsenious acid or of ammonium arsenate, of several concentrations, sometimes mixed with calcium sulfate or carbonate). The results indicated that (1) wetting with the arsenic solution a terrain containing abundant calcium carbonate resulted in the arsenic compound to remain unaltered for a long time; (2) a terrain well wet by rain caused the arsenic compound to diffuse in the ground very slowly, but not to penetrate the organs dispersed everywhere, even for concentrated original solutions; (3) it was hard to accept that a terrain containing a soluble arsenic compound was able to give up its *arsenic* to a body buried as described above. If it did, the arsenic compound should be present in every part of the body (Orfila, 1831, 1840a).

The answer to the third question relied on the possibility that the arsenic compound transformed into soluble ammonium arsenate and was carried away by the water. This was not the case when the poisoning originated from a solid arsenic compound introduced in the stomach or in the rectum, with the purpose of causing death.

Orfila concluded as follows: (1) The expert should conduct an analysis of the soil surrounding the casket only if it was damaged or the body had been buried wrapped in cloth; (2) when the corpse was integer, it should be cleaned and washed with cold water and analyzed for arsenic. In case of a positive answer and the soil did not contain an arsenic compound soluble in boiling water, the verdict should be poisoning by arsenic; (3) when the body was disintegrated and mixed with the soil, the cadaver and accompanying earth should be treated with cold water and the solution analyzed for arsenic. If the analysis was negative, it could then be assumed that the arsenic originated from the body. The ground located three or four meters from the body should also be analyzed for arsenic; and (4) if the result was negative, the cause of death was not poisoning by arsenic, except in the case where the terrain did not contain calcium sulfate, because, in general, the soluble arsenical compounds that abandoned the body, kept their ability of dissolving in cold water for a long time (Orfila, 1831, 1840a).

Blood spots

Orfila wrote that several years before, Jean-François Persoz (1805-1868) had communicated him that hypochlorous acid allowed recognizing blood spots present in a blouse having also wine and other colored spots. This acid destroyed all spots except those of rust and blood that turned brown in contact with the acid. Some tests carried by Orfila and other pharmacists had shown that the Persoz method did not seem to be appropriate for all cases of blood spots and the clothes in which they were present. For these reasons, Orfila decided to study in more detail the action of hypochlorous acid on blood spots (Orfila, 1845).

Orfila prepared the acid by the method of Antoine-Jerôme Balard (1802-1876) where well-washed gaseous chlorine was contacted with mercuric oxide diluted in water. The resulting liquid was filtrated at the end of the reaction and used as such (Ballard, 1834).

Orfila carried 38 experiments under different conditions, for example, (1) a white linen stained with vein blood was submerged in aqueous hypochlorous acid for 30 seconds; the spots became brown and remained such for seventeen hours of exposure to air; (2) the linen was stained with poppy oil and thin bloods spots and then immersed in the acid for 30 seconds. The blood spot turned brown and remained such for a long time in contact with air. Immersion of the linen in the acid for one hour caused the spot to disappear; (3) submerging in the acid for six hours an iron strip having a thin spot of blood resulted in disappearance of the spot and total cleaning of the metal, (4) submerging in the acid a line stained black by a mixture of fat and carbon did not affect the spot; (5) submerging in the acid for several seconds a linen stained red by a mixture of madder and poppy oil did not affect the stain; etc.

Orfila concluded as follows: (1) Hypochlorous acid did not present the advantages claimed by Persoz. In most cases, blood spots, thin or thick, recent or old, located in linen or iron and immersed in hypochlorous acid, disappeared almost completely after a slightly long period of time. Those that did not disappear became gray instead of red brown; (2) a short immersion, from a few seconds to one or two minutes, turned new or old blood spots brown. Nevertheless, stains caused by a mixture of fat with Sarcelles,

madder, nipplewort, poppy oil, etc., behaved like blood stains, and did not allow proper identification; (3) hypochlorous acid was completely unable of distinguishing blood stains made over linen, iron, rust, because they remained for a long time after immersion in the acid, etc. (Orfila, 1845).

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