Reflexión

Jean-Marie-Camille Méhu

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Resumen

Jean-Marie-Camille Méhu (1835-1887) fue un médico y farmacéutico francés que estudió los primeros principios en plantas, particularmente en centáurea menor y la canchalagua, y determinó que su componente activo era la eritro-centaurina; estudió los métodos para la dosificación de albúmina y encontró que el mejor se basaba en utilizar una solución alcohólica de ácido acético y fenol. También estudió y encontró el mejor procedimiento para preparar aceites fosforados de modo que contuvieran una cantidad conocida de fósforo. Méhu desarrolló una versión mejorada del lacto-butirómetro de Marchand para determinar la cantidad de mantequilla presente en la leche. Realizó un extenso estudio de la orina de origen patológico y desarrolló un método muy eficaz para separar los pigmentos coloreados, basado en la adición de una solución saturada de sulfato de amonio, etc.

Palabras clave

Aceites fosforados; albumina; centaura menor; mantequilla; eritreo-centaurin; orina.

Abstract

Jean-Marie-Camille Méhu (1835-1887) was a French physician and pharmacist who studied first principles in plants, particularly in small knapweed and canchalagua, and determined that their active component was erythro-centaurin; studied the methods for the dosage of albumin and found that the best one was based on using an alcoholic solution of acetic acid and phenol. He also studied and found the best procedure to prepare phosphorated oils, so that they contained a known amount of phosphorus. Méhu developed an improved version of Marchand's lacto-butyrometer for determining the amount of butter present in milk. He carried an extensive study of the urine of pathological origin and developed a very efficient method for separating its colored pigments, based on addition of a saturated solution of ammonium sulfate, etc.

Keywords

Albumin; butter; erythro-centaurin; phosphorated oils; small knapweed; urine.

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Life and career

The only information about the background and career of Jean-Marie-Méhu seems to be what he wrote in a booklet about his research and achievements (Méhu, 1878a).

Méhu was born in 1835; after finishing his basic education and obtaining his diploma of bachelier ès-sciences (1853) he decided to study pharmacy and within this framework he won by competition a pharmacy internship in the hospitals of Paris. (1856) and then a position of aide-pharmacien at the Pharmacie Centrale des Hôpitaux Civils (1858). The same year he joined the staff of the Hôpital de Midi. In 1862 he obtained his diploma of Pharmacist de 1er Classe and was appointed Pharmacien-chef Necker–Enfants Malades Hôpital (1862). In the meanwhile, he continued his education and obtained his diploma of licencié ès-sciences physiques (1859) and of docteur ès Médicine (1865). In 1874 he was appointed inspecteur des Établissements Classés (1874).

His scientific research earned him the first prize of the École de Médecine et de Pharmacie de Dijon (1851-1855); the first prize of the Xe Congrès Pharmaceutique (Poitiers, 1862); the first prize of the concours ouvert para la Société de Médecine du Nord (mémoire sur les liquides de la plèvre) (Lille, 1872) (Méhu, 1872); and the prestigious Montyon Prize of the Académie des Sciences (section Médecine et Chirurgie (1874), etc.

Méhu was member of the Société de Pharmacie (1864) and its President in 1878; corresponding member of the Society of Pharmacy of Chicago, Société de Pharmacie de Poitiers, Société de Pharmacie de Saint Petersburg, Torino, Warsaw, and Vienna. In 1880 he was elected member of the Académie de Médicine.

Scientific contribution

Méhu wrote more than 50 papers and books (Méhu, 1866b, 1868c, 1870bcd; 1871c, 1875a, 1878b. 1880b, 1884ab) about his research activities in organic, and inorganic chemistry, chemical synthesis, plant principles, mineral waters, etc. As customary to candidates to the Académie de Médecine, he published a booklet describing his research and achievements (Méhu, 1878a). In addition to the few subjects described below, Méhu also proposed a new method for preparing hydrogen sulfide (Méhu, 1868b), studied the composition and properties of a variety of pathologic fluids (Méhu, 1869e, 1872; 1875bc; 1877a; 1881, 1883b); analyzed the water of Santa Catalina et Guadalupe (Méhu, 1869d); studied the iron citrates and tartrates and their ammonia combinations (Méhu, 1873); proved that the liquor bismuth could be prepared with one equivalent each of citric acid and bismuth (instead of two of citric acid) (Méhu, 1874a) and that cholesterol was actually heavier than water (Méhu, 1874b); determined the composition of intestinal stones (Méhu, 1875d); developed a method to prepare mercuric sulfide crystallized by the humid way (Méhu, 1876); developed a new method for descaling copper and iron (Méhu, 1879d); studied the reaction between glycerin and certain ethereal solutions (Méhu, 1883d); etc.

Attention must be paid to the fact that Méhu used the old values for the atomic mass of the elements, for example, C = 6 (propene C_6H_6); H =1, monoatomic; and HO = water.

Small knapweed **(***Centaurium erythraea***)**

According to Méhu, little information was available about the composition of the different parts of the herb small knapweed (*Centaurium erythræa*) and the nature of its active components (Méhu, 1862abc). For example, Noël Étienne Henry (1769-1832) had investigated the composition of a water extract of the roots of yellow gentian (*Gentiana lutea*) at different periods of vegetation and concluded that the gentian herb contained (1) a particular purely vegetable matter, very similar to glue; (2) a resinous material united to a small amount of an oil, which gave its odor to the gentian. This material was also extractable by alcohol but again had no odor; (3) an extractive substance, which made up a major part of the root and was its most active component; (4) a gum united to a coloring matter; (5) a calcium-based salt, probably a phosphate; and finally, (6) the root did not contain, starch, inulin, or alkali-like material (Henry, 1819).

Henry also mentioned that Giuseppe Moretti (1782-1853) had studied the roots of the gentian and of the small knapweed and concluded that both contained a free acid, mucous matter, a bitter extracting substance (to which they perhaps owed their medical properties), and calcium salts. Only the small knapweed contained a certain quantity of oxidizable extractant, and of HCl probably combined with calcium. The results indicated that small knapweed differed from gentian in having a chloride and a greater quantity of bitter extract and oxidizable extract, while the gentian contained a greater quantity of mucous extract, different from the mucous membrane of other plants, which Moretti supposed to be the substance, which, by its decomposition, fermented and produced a spirit analogous to the eau de vie (Henry,1819).

Nicolas Deyeux (17441-1837) had written that every plant had a decided smell peculiar to itself; in some, this smell was so weak that it was scarcely perceptible. This fact had led some to classify plants into odorous and inodorous, with the first group being regarded as enjoying more properties, with more confidence placed on medicines prepared from them. This fact had suggested that waters distilled from inodorous plants had not virtue and did not differ from common water. Deyeux decided to test this assumption experimentally on 25 plants known to produce inodorous waters, among them, lettuce, argentine (*Argenteum alatum*), pellitory (*Parietaria Judaica*), orpine (*Hylotelephium telephium)*, purslane (*Portulaca oleracea*), and small knapweed. The results indicated that it was ill judged to proscribe the medicinal use of waters distilled from inodorous plants. The only problems with these waters were that they were affected by rays of light and that they should be discarded after one year (Deyeux, 1806).

The scarcity of information led Méhu to conduct additional experiments (Méhu, 1862ab). For this purpose, he carefully pulverized the plant dried in a stove and obtained a yellow green ligneous powder, almost white, smelling as the plant itself. Initial testing indicated that the best extraction procedure was based on cold distilled water, which dissolved all the tasting substances, although this required substantial amounts of water. The resulting liquor was yellow, red; its bitterness depended on the geographical location of the mother herb. It was filtrated and then evaporated, and the water vapors condensed and stored (water distillate). This concentration process led to the precipitation of a powdery material, which was separated by filtration and stored for further analysis (material A). The filtrate was extracted with alcohol of 86°. Evaporation of the alcoholic extract precipitated a bitter resinous material soluble only in alcohol. Treatment of the concentrated water

extract with ether separated a bitter principle, which Méhu named *erythro-centaurin,* a name recalling the Latin name of the plant and the red color attained under the influence of sunlight and waxy material. The procedure also separated a waxy material (Méhu, 1862ab).

The water distillate was found to be very odorant and irritant, causing lacrimation and sneezing. Drunk in small amount produced a heat sensation in the stomach. It was acid and could be neutralized without reducing its odor and irritating property. The initial water extract had none of these significant properties, they seemed to originate because of the heating process. Addition study and chemical analysis identified it as valeric acid. The water distillate was found to easily dissolve the lead of tinned vessels. Valeric acid was not responsible for the odor of the extract; the odorous principle appeared to be contained in the soft and bitter matter, which the ether removed from the aqueous extract of the plant (Méhu, 1862ab).

Méhu prepared erythro-centaurine by treating the tops of small knapweeds with cold distilled water, followed by filtration, evaporation to a syrupy state, and successive extraction with alcohol and with ether. The ethereal dissolution gave up by evaporation crystals soiled with a bitter brown material (B), accompanied by a small amount of green waxy material. The impurities were easily removed with animal black, and cleaned with HCl. The purified erythro-centaurine appeared as shiny colorless and elongated needles, which were difficult to obtain free from a yellowish tint. Microscopic examination indicated that the needles were prismatic and belonged to the clinorhombic system. It was odorless and tasteless, almost non bitter, heavier than water, very soluble in boiling water, almost insoluble in cold water, easily soluble in ether, chloroform, and carbon disulfide. Exposed to sunlight, or even to a diffused light a little bright, it turned orange, then red, and finally to bright red. Chemical analysis indicated that it was neutral and did not contain nitrogen. Méhu remarked that the overall yield of the procedure was very small, about 1/2000 (Méhu,1862ab).

Méhu treated the waxy material with a variety of reagents (i.e., alkalis, acids, etc.) and found that the alkalis did not saponify it. The wax was insoluble in water and easily soluble in alcohol and ether (Méhu,1862ab).

Material A was found partially attached to the walls of the distillation unit, while another part remained in suspension. Incineration of the first, left little cinders, while the one in suspension was found to contain 27% of calcium sulfate. Material B, treated with alcohol, gave a white, tasteless, and odorless substance, which Méhu named *centauri-retina*. This residue was a complex, viscous and colorful product. Méhu tried unsuccessfully to separate the bitter principle with a variety of solvents, among them, turpentine, and benzene (Méhu, 1862ab).

The first part of Méhu's doctoral thesis (Méhu, 1865, 1866a) repeated part of the material given in his thesis for the degree of pharmacien de 1re classe and added more detailed information about the physical and chemical properties of erythro-centaurine. The second part described the results of the same experiments done with santonin, an organic compound obtained from santonica, the unexpanded flower heads of sea wormwood (*Artemisia maritima*, var. stechmanniana).

According to Méhu pure erythro-centaurine crystals were colorless, odorless, and tasteless; neutral to colored reagents; and not hygroscopic. They melted at 136 °C; were

not volatile, and the colorless liquid, which resulted from their fusion crystallized on cooling. At a higher temperature, they turned brown, charred well, and disappeared under the oxidation fire of the torch without leaving a trace of residue. A solution of erythro-centaurine in chloroform did not deviate polarized light and was not fluorescent like esculin, quinine sulfate, etc., when placed in an environment lit by flaming sulfur. Erythro-centaurine was very soluble in distilled water at a temperature of 15 °C, and much in boiling water. It was sparingly soluble in ether and very soluble in chloroform, fatty and volatile oils, and carbon disulfide. Mineral acids increased the solubility of erythro-centaurine in water, without combining with it. Erythro-centaurine did not combine with mineral acids, alkalis, and the halogens. Nevertheless, treated in the melted state with chlorine, it went through a profound alteration. Erythro-centaurine was inert to the action of salts (i.e., silver oxide, sodium bisulfite, lead acetates, chlorides of platinum, mercury, iron, etc.) and phenol, tannin, and creosote. Chemical analysis indicated that it contained, by weight, 67.66 carbon, 5.09% hydrogen, and 27.25% oxygen, corresponding approximately to the formula $C_{27}H_{12}O_8$ (Méhu, 1865, 1866a).

Today we know that erythro-centaurine is 5-formyl-3,4-dihydroisocoumarin, $C_{10}H_8O_3$.

The most important results were related to the action of light. Pure erythro-centaurine exposed to the direct action of sunlight quickly turned pink, then bright red. When present in thick layers, only the surface turned red and acted as a screen for the underlying mass. Extensive experimentation proved that this effect was purely physical, for example, the sample had the same weight after sunstroke as it had before sunstroke; the coloring phenomenon took place in dry or wet hydrogen, hydrogen sulfide, ethylene, carbon monoxide, CO_2, SO_2, and in air. The insolated erythro-centaurine melted as a colorless liquid, which crystallized on cooling into a colorless mass; this, exposed, again it reddened, and became discolored again if its temperature was raised to 130 ºC (Méhu, 1865, 1866a).

All the above experiments on the action of light on erythro-centaurine were repeated for santonin; the results were much the same. For example, orange, yellow, and green glasses prevented the yellow coloration of santonin from occurring, and blue and purple glasses caused it. Light-yellow santonin had the same solvents as colorless santonin; dissolved in alcohol or in ether, it colored these liquids yellow, but these solutions discolored spontaneously, even more quickly in the sun than in the dark (Méhu, 1865, 1866a).

According to Méhu, his results indicated that santonin and erythro-centaurine each appear in two aspects, constituting two isomeric modifications. The two modifications of erythro-centaurine could pass from one to the other by heat, solvents, and light. But heat could not make the yellow santonin return to the state of colorless santonin; this phenomenon took place only with certain solvents (Méhu, 1865, 1866a).

In 1868 Lucien Lebeuf reported that the canchalagua of Chile (*Erythræa chilensis*) was composed, by weight, of the following materials: 7% water; 59.66% plant skeleton; 5.50% green waxy material; 2.50% chlorophyll; 9% bitter principle; 12.44 bitter oily matter (crystallized, acid black, neutral black, red colored matter, gum, and starch); and 3.90% salts (Le Beuf, 1868). These results indicated that the canchalagua contained a much larger amount of bitter principle than the 2% contained in the small knapweed, as reported by Méhu, hence, it was endowed with medicinal properties much more energetic than those of the little knapweed. To extract the bitter principle, Le Beuf treated the powdered plant with cold water for 24 hours, separated the extract by filtration, and treated the residue with

boiling water until the liquor came out tasteless. The mixture of the filtrates was treated with neutral lead acetate, and the excess of the acetate removed with hydrogen sulfide. The new filtrate was evaporated to a syrupy consistency and then treated with ether for 24 hours. The upper orange yellow ethereal layer was separated and evaporated to dryness. The residue was treated with alcohol and the filtrate evaporated to dryness. This process was repeated several times. The final yield was 9% of the original material (Le Beuf, 1868).

Méhu decided to conduct additional experiments using a small quantity of dry plants left by Le Beuf, and the same procedure he had used with small knapweed (Méhu, 1870a). The powdered plant was extracted with boiling water and the filtrate evaporated to a syrupy consistency. Mixing this liquid with concentrated alcohol resulted in an abundant precipitate, which was separated by filtration. The alcoholic filtrate was evaporated to a syrupy consistency and then mixed with ether; the resulting bitter resinoid colored mass was treated with boiling water; on cooling it precipitated colorless crystals easy to separate mechanically from the greater part of the resinous material, which accompanied them. These crystals, purified by recrystallization from chloroform, were found to be identical with erythro-centaurine, as shown by its melting point (136 °C) and other properties (Méhu, 1870a).

Dosage of albumin

Méhu wrote that various methods were available for detecting and quantifying the amount of albumin present in physiological or pathological fluids, particularly, urine. These methods were based on the ability properties of albumin to coagulate when the temperature was increased, to precipitate under the influence of various reagents, sometimes by forming insoluble combinations with them (i.e., tannin and potassium ferricyanide), sometimes by becoming simply insoluble in the precipitating liquids (i.e., alcohol, phenol, and nitric acid), and finally, on being levorotatory (Méhu,1869bc). The commonly used methods were (1) Berzelius' method, based on coagulation by heating to almost boiling (Berzelius, 1828), (2) coagulation with nitric acid, and (3) Bödeker's method, based on coagulation by acetic acid and potassium ferricyanide (Bödeker, 1859).

According to Méhu, none of the methods available was sufficiently reliable, each one had advantages and disadvantages, and required careful treatment to reach their goal. For example, blood albumin coagulated at 72 °C in flakes or in a compact mass according to the quantity of albuminous principle; the presence of certain chemicals resulted in an increase (i.e., acetic acid or phosphoric acid) or a decrease of this temperature (i.e., sodium sulfate or chloride). Before coagulation, required the liquid to be perfectly clear and acidic. Certain body fluids, like pathological urine, were usually basic, this required adding to it drop by drop diluted acetic acid until the liquor showed a very slight acid reaction, otherwise certain substances (i.e., mucus, urates, and phosphates) would remain in suspension or cause a partial precipitation of the albumin. Usually, washing of the precipitate was difficult, slow, and incomplete; the dry precipitate did not always correspond with sufficient accuracy to the real quantity of albumin. Coagulation of a very concentrated albuminous liquid resulted in the solidification of the albumin, making it difficult to divide and wash with water. Acetic acid was usually added to decompose the albuminous matter in combination with the alkalis, to liberate the albuminous matter, to lower its point of coagulation, and to make it complete. Addition of too much acetic acid to the albuminous liquid resulted in the loss of its ability to heat-coagulate (Méhu, 1869bc).

Nitric acid was known to coagulate albumin without combining with it, but it was unable to precipitate all the albumin dissolved in a liquid, even when added in large excess. Using a small quantity of nitric acid required the additional use of heat to complete the precipitation. Heating for a long time resulted in the acid reacting with the precipitate, turning it yellow, and transforming it into xanthoproteic acid. Cold nitric acid, concentrated enough to precipitate all the albumin, reacted in the same way, and finally dissolved the coagulated albumin or the yellow products of its transformation. The yellow precipitate turned brown during its desiccation, and, if it was collected on a filter, it was present at very variable weight, and often did not even reach half the weight of the albumin, which had been added to the mixture (Méhu, 1869bc).

This information led Méhu to search for a better procedure for determining the amount of albumin present in a liquid, based on the known fact that phenol, creosote, and various other substances coagulated albumin and avoided its putrefaction. Early experiments showed that the solubility of phenol in water was not enough to cause the precipitation of albumin. This disadvantage was easy eliminated by dissolving the phenol in three or four times its volume of alcohol; unfortunately, this mixture precipitated the albumin together with most of the mineral salts, particularly the alkaline earth phosphates. Additional testing proved that this problem could be solved by adding a certain quantity of acetic acid to the alcoholic solution of phenol. Extensive testing conducted at the Necker hospital using a synthetic mixture of albumin of the egg or of the serum, proved that the best proportion of the three substances was one part of phenol, one part of commercial acetic acid, and two parts of alcohol of 86°. This solution could be kept indefinitely without any alteration. In addition, phenol was found not to react with albumin (Méhu, 1869bc).

The basic process was as follows: 100 g of the albuminous liquid, urine for example, were mixed, successively and with stirring, with 2 cm^3 of ordinary nitric acid and 10 cm^3 of the phenolic solution. The precipitate was separated using a very dry white paper, of known weight, followed by washing with water containing 0.5% phenol, and finally, with slightly alcoholic water. The loaded filter was dried at 110 °C and weighed between two watch glasses after cooling, over sulfuric acid. The difference in weight provided the weight of albumin present in the primitive liquid. Addition of diabetes sugar, magnesium sulfate, potassium iodide, potassium nitrate, sodium chloride, etc., did not change the results. Méhu illustrated the use of his procedure in the case that the original liquid was egg white, thoracentesis fluid, and ascitic fluid. Méhu added that his process could also be used to recognize faint traces of albumin and it was more sensitive than nitric acid and heat. It also required only a very small number of simple instruments: a precipitate glass, a small glass funnel, and a good balance. (Méhu, 1869bc).

Phosphorized oil

Méhu wrote that in the last years, solutions of phosphorus in oil had become more popular for treating hard diseases such as paralysis (Méhu, 1868a). The French Codex of 1866 indicated that this medicine should be prepared by dissolving 2 g of phosphorus in 100 g of sweet almond oil heated in a water bath and left to cool after dissolution. The solution was separated by decantation from the phosphorus crystallized at the bottom of the flask, and then stored in small capacity flasks. According to Méhu, the recipe left much to be desired because the final product had not a constant composition. The phosphorus

dissolved in variable quantities according to the temperature, the amount of phosphorus that separated by crystallization, the degree of purity of the oil, and its age. It could well be that the medicine was put in use before all the undissolved phosphorous had precipitated. In addition, experience had shown him that the dissolution of phosphorus in ordinary sweet almond oil was always accompanied by a reaction between the organic substances (albumin, resins, etc.), which were part of the natural oil (Méhu, 1868a).

After much testing, Méhu believed that had developed an improved procedure, which seemed to obviate the shortcomings and produce a clear and unalterable phosphorus oil. In this technique, the very clear sweet almond oil was heated in a porcelain capsule for about a quarter of an hour at a temperature of 150 °C, and then for about ten minutes at a temperature of 200° to 250 °C. This resulted in the release of water vapor, accompanied by the destruction or volatilization of some easily alterable organic matter, and the oil becoming completely discolored. The next step was preparation of the phosphorus oil. This was done by adding to the oil 0.01 g of phosphorus for each gram of oil. This phosphorus had to be very transparent and free from red and white phosphorus. Comparison of this new method with that of the Codex indicated that heating the oil at high temperature eliminated the foreign substances that could deteriorate it, and that the two components were present in a known ratio. Méhu added that the same procedure was applicable to olive oil and white oil.

Méhu observed that oil containing 1/000 phosphorus was phosphorescent in the dark. This property disappeared when the concentration was raised to 2/1000, and upon addition of certain solvents such water, carbon disulfide, ether, turpentine, etc., and a variety of essences. Méhu suggested that the method described in the Codex of 1866 be replaced by the one developed by him (Méhu, 1868a).

In a following paper, Méhu extended his results to many other oils, based on the facts that not all fatty oils could withstand 250 °C, without deterioration (i.e., brown cod liver oil and hemp seed oil), and not all oils that were overheated would discolor as easily as almond oil, but in this case, exposure to direct sunlight would probably achieve de goal (linseed oil, for example). Discoloration was not a condition for the phosphorized oil to keep well, it was enough that it had been superheated to 250 °C. Preservation of the oil was achieved by the destruction of some very alterable organic elements, not by bleaching (Méhu, 1869a).

Méhu found that the essences that prevented phosphorescence of phosphorus oil included bergamot, lemon, copahu, lavender, mace, thyme (liquid part), mustard, rosemary, turpentine, and mint. All these essences were characterized by the fact that they did do not contain oxygen in their composition. In the same manner, essences that did not prevent phosphorescence or which had this power only to a low degree, included anise, lemongrass, bitter almonds, fennel, cajeput, geranium, chamomile, cloves, Ceylon cinnamon, bay leaf, cherry, wallflower cinnamon, Rhodes (wood), sandalwood, sassafras, and verbena (Méhu, 1869a).

Butter dosage

According to Eugène Marchand (1816-1895), the lacto-densimeter of Théodore-Auguste Quévenne (1806-1855) (Quévenne, 1841) and the centesimal galactometer of Étienne Ossian Henry (1798-1873) and Alphonse Chevallier (1828-1875) (Henry & Chevallier, 1839) were appropriate for analyzing milk pure or diluted with water but failed if the milk examined had been partially skimmed. In 1854 he presented an improved version of the

apparatus, based on the following facts (1) small quantities of caustic alkali did not saponify fatty bodies in the presence of glucose, lactose, or casein; this process took place after the lactose or the glucose had been transformed into a brown material and the casein into a thick mucilagous substance; (2) the large solubility of butter in ether, even in presence of water; and (3) the very low solubility of fatty bodies in a liquor composed of equal volumes of ether, alcohol, and an aqueous solution of lactose or casein, such as skimmed milk (Marchand, 1854). The basic idea relied on the fact that mixing equal volumes of milk and ether resulted on the dissolution of the butter in the ether. Further addition of a volume of alcohol equal to that of the ether, separated the butter as a floating oily layer. Conducting these operations in a graduated tube allowed reading directly the quantity of oily material separated, which was in a constant ratio to the butter contained in the milk tested. Addition of a small amount of NaOH avoided the partial coagulation of the casein, which opposed the complete separation of the butter. Marchland's lacto-butyrometer was so successful that it became a standard instrument in all French military and civil hospitals, as well as with chemists. It was examined and approved by the Académie de Médicine, who recommended its adoption by the Ministries of Agriculture and of Justice. (Marchand, 1854).

As a member of the Necker hospital, Méhu was forced to use Marchand's method. This led him to identify some of its shortcomings and propose ways for improving it (Méhu, 1870). According to his experience, Marchand's procedure was very valuable when applied to fresh milk, but not do for dairies located far away, particularly in summer. The obligation to maintain the casein in solution with one or two drops of NaOH sometimes resulted in no trace of free butter being obtained, while the same milk tested without the addition of this alkali gave a sufficient proportion of butter to remove any suspicion of fraud. Several of Méhu hospital colleagues had also noticed that the milk tested with the addition of NaOH sometimes gave no trace of free butter, while without this alkali, a significant amount was separated.

Méhu main modifications included (1) using Marchand's graduated tube, with dry ether and alcohol of 90°, cold saturated with crystallized boric acid; (2) no addition of caustic soda, (3) vigorous stirring, to separate the casein into flakes of a great tenacity, which settled rather quickly at the bottom of the tube without noticeably hindering the separation of the butter, and (4) closing the tube with a good stopper and keeping it in another larger tube containing water at 36 °C. This resulted in a clear separation of the butter (Méhu, 1877c).

Urine

Méhu studied the properties and behavior of urine in healthy and different pathological situations, as a typical body fluid (Méhu, 1871ab, 1877b, 1878, 1879ab, 1880a; 1883ac).

In his first paper, he wrote that urine with blue sediment was rarely observed free in some pathological urine and had so far been studied only partially. Many times, this blue matter was accompanied by a red matter and together, they tinted urine purplish. Urines with purple sediment were usually shown in patients suffering from generally serious affections, mainly of the spinal cord. Méhu mentioned that care should be taken not to confuse these urines with those, which resulted from the action of concentrated HCl on ordinary urine (Méhu, 1871a).

At one time, Méhu had the opportunity to study a urine poor in coloring matters (blue and red), which gave no indication of forming a purple sediment. This urine was

slightly albuminous, extremely alkaline, and of a most fetid odor. It was slightly colored violet by HCl. Méhu tried unsuccessfully to extract the coloring matter with lead oxide and succeeded only when using a mixture of ether and chloroform. By evaporation and a series of other operations he obtained the mixture of coloring matters freed from the fats, resinoids, etc., which normally accompanied them. He then separated the blue matter from the red one by slow evaporation of their alcoholic solution at room temperature. The blue matter (indigotin) deposited, adhering enough to the walls of the vessel from where it was easily freed means of ether or chloroform. The resulting impure red matter (indirubin) was insoluble in water, soluble in ether, alcohol, chloroform, and ammoniacal water. The blue matter was also insoluble in water, less soluble in alcohol than red matter, and dissolved with difficulty in chloroform and ether. Méhu wrote that indigotin was known to be insoluble in alcohol, ether, fatty and volatile oils, diluted acids, and alkalis. Concentrated and boiling ethanol and methanol dissolved enough, however, to become distinctly blue in color. Méhu found that indigotin dissolved in phenol, particularly when hot. On cooling, the indigotin separated in crystalline form. Crystallization of the phenol could be prevented by adding camphor and benzene (Méhu, 1875ab).

In another publication, Méhu remarked that the common method for extracting the coloring matter from these substances involved using lead salts at appropriate temperatures. These conditions were known to be defective, often giving reaction products very different from the natural coloring principles. He now had found a new method, which allowed extracting most of the animal coloring principles soluble in water, at room temperature and without alteration. The procedure involved using ammonium sulfate, often slightly acidified with sulfuric acid (Méhu, 1878).

It was known that certain pathological conditions of the liver expressed themselves in the effluent urine being tinted orange or red, evoking urine loaded with the coloring principles of rhubarb. Very commonly, this urine was erroneously assumed to be icteric urine charged with bilirubin. A simple test with nitric acid would discard this possibility; the action of the acid would not show the color of the urine going through the succession of colored hues green, blue, violet, reddish, orange yellow (known under the name of Gmelin reaction) but turning it dark red or old mahogany. Méhu named this urine *red hepatic urine* to distinguish it from red bloody urine and orange urine containing bilirubin (Méhu, 1878). In this situation, the red pigment was separated by first acidifying it with about 2 g of sulfuric acid per liter of urine, followed by saturation with ammonium sulfate in slight excess. The precipitated pigment was separated by filtration, washed with a saturated solution of ammonium sulfate, and then dissolved with alcohol of 95%. The alcoholic solution was orange-yellow or red depending on the proportion of the coloring matter. Spectroscopic examination of this solution gave an absorption band, which made the blue (the neighboring parts of Fraunhofer's *F*-ray) disappear. Mixed with dry zinc chloride, and then filtered, the alcoholic pigment solution of hepatic red urine was transparent and pinkish red when seen by transmission, and opaque and of an intense green by reflection. The pigment was easily characterized by the dichroism of its alcoholic solution mixed with zinc chloride. The red pigment was separated by saturating the alcoholic solution with ammonium sulfate; it was soluble in water and in amyl alcohol. Bile salts and sodium phosphate increased its solubility in water (Méhu, 1878).

In a following paper, Méhu indicated that treating urine by his method resulted in the immediate separation of the pigments urobilin, bilirubin, biliverdin, etc., which were insoluble in the liquid saturated with ammonium sulfate. He now recommended using sulfuric acid of 1% instead 2%. The resulting precipitate was separated by filtration over a white filter paper, followed by washing with a small quantity of a saturated aqueous solution of ammonium sulfate, and drying in air or in an oven at low temperature. The different pigments were then separated with the appropriate solvent. This method was equally applicable to a red matter (indirubin, as described above), or to blue one (indigotin, as shown below) (Méhu, 1883a).

The resulting solid residue filter was purplish; it retained ammonium sulfate, sometimes albumin, urates, and various anatomical elements, and finally, the always very minimal quantity of blue and red coloring matters. The separation of indigotin from indirubin was easily achieved with alcohol of 50%, a solvent that dissolved indirubin completely and left a dry filter, intensely blue. Indigotin could then be extracted with phenol melted at high temperature. During the slow cooling of this liquor, part of the indigotin deposited in well-defined crystals, while the part of the indigotin in solution colored the solution intense blue. This permitted calculating the amount present by comparing the intensity of the color with that of synthetic mixtures of known composition (Méhu, 1883a).

Méhu found that phenol could be substituted by several other solvents, such as beech creosote, essence of anis, essence of wintergreen, and amyl alcohol. Turpentine and rosemary were poor solvents (Méhu, 1883a).

Méhu also dealt with the opposite problem: the redissolution of the pigments, an operation useful above all for facilitating the examination of urinary sediments laden with urobilin, uroerythrin, etc. (Méhu, 1883c). This task could be easily conducted using an aqueous solution of ordinary drugstore sodium phosphate, 2NaO,HO,PO5,24HO. This cold saturated solution dissolved with the greatest ease urobilin, uroerythrin, ordinary bile pigments (bilirubin, biliverdin), etc. For doing this, it was enough to add to the cold liquid a few drops or a few grams of the sodium phosphate solution. The redissolution of the pigments and urates was almost immediate. An excess of reagent did not have any disadvantage. (Méhu, 1883c).

In 1854, Edmond-William Davy (1785-1857) found that urea was readily decomposed by the chlorides, particularly by the hypochlorites of sodium, potassium, and calcium, according to the equation:

$$C_2H_4N_2O_2 + 3(NaO, Cl, O) = 2CO_2 + 4HO + 3Cl,Na + N_2$$
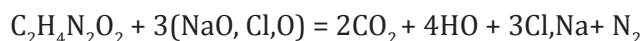
This fact suggested dosing urea by simply measuring the volume of the nitrogen released, due care taken of the temperature and atmospheric pressure. Davy preferred sodium hypochlorite because it was easily procured and in a high degree of purity. He suggested that this chemical be used in excess to assure the full execution of the reaction (Davy, 1854). Later, in 1858, Charles Leconte and others noted that sodium hypochlorite released, when hot, only 34 cm^3 of nitrogen, at a 0 °C and 760 mmHg, instead of 37.3 cm^3 indicated by the theory (Leconte, 1858). Substitution of sodium hypochlorite by sodium hypobromite did not solve the problem. Consistently, several other researchers (including Méhu), found that the volume of nitrogen released was about 8% lower than the theoretical value.

Méhu mentioned that a long series of observations had led him to believe that the urine of diabetics gave a more complete nitrogen yield than the urine not loaded with glucose (Méhu, 1879ab). For this reason, he decided to compare the nitrogen yields of two solutions containing strictly the same weight of urea in the same volume of liquid, one of them prepared with pure water, the other with water charged with glucose or cane sugar. The results confirmed his assumption: the sugary urea solution yielded 1/14 more nitrogen. Not only that, but the volume of nitrogen released by the sugary urea solution was completely in agreement with the theoretical value, while that of the unsweetened was again about 8% lower, all temperature and pressure corrections being made. These results indicated that to conduct Davy's method it was necessary to add sugar beforehand to a determined volume of this urine before reacting it with sodium hypobromite (Méhu, 1879ab).

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