RESUMEN. Jules-Antoine Regnault (1822-1895), médico y farmacéutico francés que realizó investigaciones sobre los alcaloides midriáticos (como la atropina), fenómenos electroquímicos, fuerza electromotriz, amalgamas, propiedades de mezclado en disoluciones salinas, fisiología, electrofisiología, fluorescencia de órganos vivos, química médica, anestesia y fenómenos de visión. Como resultado de ellos inventó un nuevo procedimiento para medir la fuerza electromotriz (método de oposición), y desarrolló una explicación de la influencia de la electricidad sobre los músculos y la visión binocular.

ABSTRACT. Jules-Antoine Regnault (1822-1895), French physician and pharmacist, who carried researches about mydriatic alkaloids (such as atropine), electrochemical phenomena, electromotive force, amalgams, properties of mixing in saline solutions, physiology, electrophysiology, fluorescence in live tissues, medical chemistry, anesthesia, and vision phenomena.

Life and career\textsuperscript{1,4}

Very little information is available about the early life and education of Jules-Antoine Regnault (Figure 1).
He was born in Paris on November 26, 1822, the son of a pharmacist, who died in 1839 leaving a very modest fortune to his wife and children. According to Charles-Jules-Ernest Cadet de Gassicourt (1826-1900) this two harsh events turned Jules into the introvert person he was known for his whole life. Under these circumstances, Jules decided to follow the profession of his father and take an apprenticeship in a Parisian pharmacy and hospital pharmacies. In 1844, he became hospital pharmacist and took a position at the Hôpital des Cliniques (1844-1855), while simultaneously following medical studies at the Faculté de Médecine de Paris, from where he graduated in 1847, after successfully defending a thesis about the generation of electricity in organized entities. In 1844, he was elected member of the Société de Pharmacie. He continued his education at the Faculté des Sciences de Paris and received his degree of docteur-ès-sciences (1851) with a thesis about electromotive forces and a new method for their measurement. In 1856, he began working at the Hôpital de la Charité in Saint-Etienne (1856-1859) and in 1857 he won an aggregation contest, which allowed him to follow an academic teaching and research career. Thus in 1857 he was appointed professor of physics at the École Supérieure de Pharmacie, replacing Eugène Souberain (1797-1859), his father-in-law; followed by an appointment to the chair of pharmacology at the Faculté de Médecine (1859-1891). In 1859, he was appointed director of the Pharmacie Central des Hôpitaux Civils, a position he kept until 1876 [Marie-Victor Ernest Beaudrimont (1821-1885) replaced him]. In 1861, he was elected member of the Académie Nationale de Médecine (section of physics and medical chemistry), of which he became its vice president in 1891 and president in 1892. Jules-Antoine Regnauld passed away in Paris, on February 9, 1895, after being ill for some time. His wife and only son, Eugène, survived him. By his express wish no official delegation was present at his funeral and no eulogies were pronounced. Paul Regnard (1850-1927) replaced him at the Académie de Médecine.

SCIENTIFIC WORK

Regnauld published about 60 papers, booklets, and books describing the results of his research on amalgams, anesthesia, batteries, electrophysiology, ether, medicine, physiology, vision phenomena, and phenomena of mixing of solutions. As customary for all candidates to the Académie Nationale de Médecine, he published a booklet describing his researches and achievements.

Vision phenomena

In 1838, Charles Wheatstone (1802-1875), in a groundbreaking paper, reported some new phenomena of binocular vision. According to him, "when an object is viewed at so great distance that the optic axes of both eyes are sensibly parallel when directed towards it, the perspective projections of it, seen to each eye separately is similar, and the appearance to the two eyes is precisely the same when the object is seen by one eye only...This similarity no longer exists when the object is placed so near the eyes that to view it the optic axes must converge; under these conditions a different perspective projection of it is seen by each eye, and these perspectives are more dissimilar as the convergence of the optic axes becomes greater." In particular, Wheatstone remarked "that if a blue disk was presented to the right eye and a yellow disk to the corresponding part of the left eye, instead of a green disk which would appear it these two colors mingled before their arrival at a single eye, the mind will perceive the two colors indistinctly one or the other alternatively predominating either partially or wholly over the disc." Wheatstone explained this phenomenon by assuming that the retina contained two compartments and the papilla present in each unit was excited simultaneously. These results could be easily observed using the stereoscope apparatus of Wheatstone's invention.

According to Jean Bernard Foucault (1819-1868) and Regnauld, the majority of researchers who had investigated the phenomena of visualization of rays of different refrangibility, had
reached the opposite conclusion, that is, the visual appearance would be a mixture of the two colors. For these reason they decided to repeat and extend Wheatstone's experiments, using the stereoscope of his invention because "it offered a simple mean of eliminating all possible complications which would affect the correctness of the physiological results".21

The experiments were conducted as follows: Two plane mirrors forming a variable dihedral angle were attached to the stereoscope and their vertical edge was placed symmetrically in relation to that of the two glasses of the stereoscope. Two large circular apertures perforated the vertical supports, which housed the grooves for introducing the image. The two grooves carried glasses, each pasted with identical circular screens of white paper, and of a diameter less that of the apertures. Two large complementary rays, obtained by chromatic polarization, were directed horizontally on the plane mirrors, which reflected them on the circular screens. This produced two colored discs, which become the images conveyed by the stereoscope to the corresponding elements of the retinae. Proper appropriate disposition of the polarizing apparatus allowed successively passing numerous complementary tints, while varying at the same time the intensity of the two colored images and the intensity of one or other of the images separately.

Foucault and Regnauld reached the following conclusions: (1) When the corresponding elements of the retinae were excited simultaneously, the alternations of activity or inertness of one of the eyes was generally perceived at the initiation of the experiment; occasionally one of the tints was perceived, and at other times its complementary one; but after an interval, which varied very much in different individuals, only a single white circle was seen; (2) When the eyes were in some degree accustomed to this unusual mode of impression, the tendency to recomposition of the light became so strong in some persons that the screens might present successively all the complementary tints which the apparatus furnished without there being any sensation corresponding to the colors; the white light alone was seen; (3) On diminishing the intensity of one of the colors, the other remaining constant, recomposition still took place; but the white disc appeared to become covered more or less strongly with the predominant color; (4) When the intensity of the two complementary rays was varied in the same manner for the two ray beams, the recomposition took place more easily at the beginning of the observation, as their intensity was more moderated; (5) Of the complementary rays examined, the sensible blue and the yellow tints were best adapted for the experiment and immediately furnished the sensation of white. To Foucault and Regnauld this result was due to easy of accommodation of the eyes to the portions of the spectrum occupied by these rays; and (6) Except for few exceptions, the sensation of white light could be produced by any two complementary chromatic impressions in each of the eyes; that the sensation solely of white arising from two complementary rays was independent of any mutual action of these rays externally to the visual apparatus and that the luminous impressions produced on the retinae retained their properties even to the innermost recesses of the brain.21

Fluorescence of the transparent media of the eye

According to Regnauld, it was well known that the luminous radiations emanating from the sun, from a fire, or from an electric battery, contained three sorts of rays in different proportions, viz., luminous, thermal, and chemical rays. The chemical rays were particularly abundant in the violet, ultraviolet, and higher portions of the spectrum. It was also known that bodies such as quinine sulfate, uranium glass, and many others, had the property of fixing these rays, that is, absorbing them instead of reflecting or permitting them to pass by and thus becoming luminous of themselves. This property of fixing these chemical rays was named of *fluorescence*, and the bodies possessing were called *fluorescent*.22,23

Regnauld found that many constituent parts of the eye became fluorescent under the influence of ultraviolet radiation, which he separated by means of a very pure and large Nicol prism. By successive inclination of the prism it was possible to receive over a screen, parallel or divergent rays of refrangibility increasing from blue to ultraviolet and non-visible. In this zone, a very
fluorescent substance such as a plate of uranium glass, revealed the existence of a dark field, where the tested material (e.g. transparent cornea, crystalline, hyaloid body, and retina) should be located. Since it was not possible to report the intensity of the phenomenon in relation to an invariable unit, Regnauld chose to compare it to the fluorescence of ordinary glass tubes illuminated by the long sparks transmitted through a rarefied gas.22,23

His results indicated that (a) in man and mammals the cornea fluoresced in a very slight degree; (b) in man and mammals (e.g. the sheep, dog, cat, and rabbit) the crystalline lens possessed the highest degree fluorescent properties. In these animals, and also in many birds, the central part of the lens [the endophaine of Pierre Henry de Valenciennes (1750-1819) and Edmond Frémy (1814-1894)] retained this property even after being desiccated at a low temperature; (c) the central portion of the crystalline of many aquatic vertebrata and mollusks (the phaconine of Valenciennes and Fremy) was almost entirely without fluorescence; (d) the hyaloid body was weakly fluorescent. The phenomenon seemed to be due to the hyaline membrane because the vitreous humour was not fluorescent; (e) the retina of a human eye retained, even eighteen hours after death, certain fluorescence not at all comparable in intensity to that of the crystalline lens of mammals, as Hermann von Helmholtz (1821-1894) had reported previously; and (f) Regnauld believed that if we must attribute the accidents caused by weakly luminous radiations of the electric light to the phenomena of fluorescence, it is above all in the energetic action on the crystalline that it is natural to look for an explanation. The impression, which the cornea experimented, must nevertheless not be neglected.22,23

Regnauld finished this paper with some remarks regarding the safety of the vision process: The fluorescent properties of the cornea and the crystalline provided an almost impassable obstacle to the chemical rays inappropriate for the vision process and those dangerous to the retina. This effect was particularly important when the eye was subjected to an abundant flux of ultraviolet rays, as in the case of light produced by electricity. For this reason the present efforts to introduce electrical lamps for the illumination of cities and towns was fraught with dangers; the use of this type of light could have a slow and deadly effect on the eyes of the inhabitants.22,23

Mixtures of saline solutions

Refractive index

Regnauld published several papers reporting certain changes that took place during the mixing of saline solutions of different composition and nature.24-28 The first one studied the possible changes of the average refractive index that occurred when mixing two aqueous solution of known refractive index, which did not go through a double decomposition, in a given proportion.24 The solutions were sufficiently diluted to remain as such after their mixing. Regnauld did not make use of the direct value of the refractive indexes but of a simple procedure, which indicated the nature and direction of the change. For this purpose he used the apparatus designed by Gustav Robert Kirchhoff (1824-1887) and Robert Wilhelm Bunsen (1811-1899),29 where the flint prism of the photometer was replaced by a hollow glass receptacle, built of parallel blades in a way to create a prismatic cavity of fixed angle. Regnauld used this apparatus to examine the changes in refractive index when mixing solutions of sodium acetate with solutions of zinc (or copper) sulfate, nitrate, and copper, and of sodium formate with the same salts of zinc or copper. The compositions of both the solutions being compared were adjusted by dilution so as to produce the same refractive index relative to the D line. This procedure assured that the mass ratio of the two salts was constant during the standardization process.

Regnauld found that mixing a solution containing a strong acid and a weak base with another having a weak acid and a strong base produced a resulting solution having a lower index of refraction. According to Regnauld, since the mass ratio of the solutes was constant implicated that the groups of the salts had rearranged themselves in a different manner. To confirm the correctness of his hypothesis he carried the inverse experiment, that is, mixing a solution of
copper or zinc acetate, with another of sodium nitrate or sulfate, etc. In this situation, the mixture of the solutions could lead to the formation of salts resulting from the union of a weak acid with a weak base, and the opposite effect would be observed: an increase of the refractive index. The result of these experiments confirmed his theory.24

In the second part of this work Regnauld repeated the experiments using a variety of pairs of additional salts of potassium, calcium, ammonia, copper, zinc, nickel, manganese, cadmium, and iron, selected in such a way that their mixing occurred without chemical reactions. Once again, he observed a decrease in the refraction index when the original solutions contained a strong acid combined with a weak base or a strong base combined with a weak acid, or an increase in the refractive index when the mixture of the solutions could lead to the formation of salts resulting from the union of a weak acid with a weak base.25

Regnauld also measured the density of the solutions, before and after mixing them, and noted that pairs that produced a decrease in the refraction index were accompanied by an increase of volume, while those who produced an increase in the value of the index exhibited a decrease in the volume.25

**Heat of dilution**

It was thought that the heat effect during the dissolution of a salt should be less than the heat necessary to melt it because the chemical interaction between the salt and the water provided the part of the heat necessary for melting the salt. In 1850, Charles Cléophras Person proved that this conception was wrong.30 For example, he mentioned that 49 calories were necessary for melting one gram of potassium nitrate, 69 calories for dissolving it in 5 g of water, and 80 calories for dissolving it in 20 g of water. This was the common result for salts that showed little affinity for water: the heat effect increased with the amount of water employed, although not proportionally. Salts showing a large affinity for water showed the opposite effect. Thus, under the same conditions as above, calcium chloride required 41 calories to melt and only 20 for dissolution. All kinds of phenomena occurred in between these two cases, potassium phosphate, for example, required the same amount of heat for melting and for dissolving in water. Person wrote that these and other experiences showed that it was necessary to accept the existence of a heat of dilution.30

Regnauld went on to prove that the variation of heat observed by Person during the dilution of a salt was not related with the accompanying change in density of the solution.26 For this purpose he prepared a saturated solution of nine different salts at 15 °C, then mixed each suddenly with an equal volume of water, observed the direction of the temperature change, then let the liquid achieve thermal equilibrium and measured its density at 15 °C. The results indicated that the dilution process of solutions of sodium nitrate, potassium nitrate, sodium hyposulfite, and sodium sulfate was endothermic, while for those of sodium acetate, zinc acetate, and zinc sulfate, was exothermic. The solution of sodium phosphate showed no change in temperature. A comparison of the calculated density (weighted average of that of the components) was always less than the experimental one. In other words, the actual effect was one of contraction, independently of the thermal effect.26

**Volume changes**

In a following publication Regnauld reported additional data about the changes of volume that occurred during the isothermal (15 °C) neutralization of alkaline solutions with acids. In this opportunity his experimental technique consisted in neutralizing equal volumes of solutions containing one equivalent of base (KOH, NaOH, baryta, and ammonia) or one equivalent of acid (sulfuric, nitric, phosphoric, chlorhydric, acetic, and tartaric), and measuring the density of each solution and that of the resulting one. He determined the change in volume (positive or negative) by comparing the latter result with the theoretical density [calculated as (d₁ + d₂)/2] 27
The experimental results indicated that independently of the dilution and the nature of the acid that combined with NaOH, KOH, and baryta, the neutralization process was always accompanied by an increase in volume (expansion). The opposite result, contraction, was obtained with ammonia. Interesting enough, both phenomena were accompanied by a strong exothermic effect. Regnauld believed that the difference in behavior originated from the role played by the water in both types of basic solutions. In spite of its solubility in water, ammonia did not produce a stable combination with the elements of water; the gas dissolved according to the laws of gas solubility and escaped under vacuum, heat, or by simple diffusion into the atmosphere. Without pre-assuming the existence of a hydrate of ammonium oxide, it could be said that this liquid behaved in the same manner as if the elements of NH₃ were condensed physically by water, as they were by carbon (adsorption), with the corresponding exothermic effect. KOH and NaOH represented a completely different picture; the new structure was built at the expense of materials solidly united. These hydrates had long been likened to salts and their production was always accompanied by contraction and heat release.²⁷,²⁸

Electrochemistry

Regnauld wrote that famous scientists such as Georg Ohm (1789-1854), Gustav Fechner (1801-1887), Johann Christian Poggendorff (1796-1877), and James Prescott Joule (1818-1889), had developed methods and apparatus for measuring the electromotive force, which unfortunately were all indirect. The value of the force was calculated from the intensity of the current, or was implicitly included. In addition, all the methods required knowledge of the resistance of the circuit. In a series of papers he described a new method (which he named the opposition method) for measuring the electromotive force, based on the cancellation of the resistances. The values were determined directly, without going through the intermediate steps of measuring the resistances or the intensities.³¹-³³

The bases of the new method were as follows: Assume two voltaic cells composed of the same elements but of different size, that is, the metallic plates do not have the same dimensions, the liquid layers have different depth or section, etc. Under these circumstances each cell will produce a current of different intensity. If, for example, the positive element of one cell were connected to the positive element of the other cell, and the same was done with the negative elements, then no current would be produced. If the same arrangement were built with cells of different nature, a current would be generated having intensity equal to the difference of the intensity produced by each cell [Regnauld remarked that Michael Faraday (1791-1867) had mentioned these ideas but never formulated them explicitly].³¹-³³

Assume now that the intensity of the two cells is expressed by \( e_1/r_1 \) and \( e_2/r_2 \), respectively, where \( e_i \) represents the electromotive force and \( r_i \) the resistance. If the cells are connected as described above, the net electromotive force will be given by the difference \( (e_1 - e_2) \) but the resistance of the circuit will be \( (r_1 + r_2) \); hence the intensity \( i \) of current will now be given by

\[
i = \frac{e_1 - e_2}{r_1 + r_2}
\]

Equation 1 shows that if \( e_1 = e_2 \), no current will flow, independently of the value of \( (r_1 + r_2) \).

The same reasoning may be applied to series of cells in opposition, that is

\[
i = \frac{\sum e_i - \sum e_i}{\sum r_i + \sum r_i}
\]
Hence, if a sensitive galvanometer is located in the circuit, a reading of zero will indicate equilibrium of the electromotive forces. According to Regnauld the only thing missing to compare different cells was to select one of them as the unit of reference; it should be a couple having absolutely constant behavior and an electromotive force sensibly smaller from that which was to be measured. For this purpose he selected the couple bismuth-copper, for a temperature difference between 0 and 100 degrees. The electromotive force of a wet cell was measured using as unit standard, a Daniell cell in which the copper and the cupric sulfate were replaced by cadmium and cadmium sulfate.31-33

Regnauld gave a detailed description (including drawings) of the construction and operation of the circuit, as well as of the bismuth-copper reference couple. He also included the results of his measurements for a large number of wet cells.31-33

In 1856, a memoir from Victor Doat was read to the Académie des Sciences, describing a new pile of constant current consisting of a modified cell in which mercury had replaced the zinc of ordinary cells, and a solution of KI the acidulated water or NaCl. In the case of two-liquid cells, the nitric acid or the cupric sulfate was replaced by iodine dissolved in KI. In all cases, carbon constituted the negative pole.34 Shortly thereafter Regnauld published a paper in which he reported the measurement of the electromotive force of the Doat cell and other similar cells, using his opposition method. He indicated that the electromotive force of the Doat cell was 102 units, compared with 175 for the Daniell cell and 310 for Grove's cell. He reported that the electromotive force of the Doat cell could be increased substantially if the mercury was replaced by cadmium (182 units), zinc (216 units), sodium amalgam (381 units), or a potassium amalgam (386 units). Higher values were also obtained when the halogen iodine was replaced by bromine or chlorine: 471 units for bromine-potassium amalgam and 512 units for chlorine potassium amalgam.35

In another publication Regnauld discussed the use of his method of opposition to measure the electromotive motive force associated with muscular currents.36 Previous researchers had reported serious polarization problems, when measuring this property using platinum strips submerged in a solution of sodium chloride. Regnauld solved this problem by replacing the platinum by zinc purified by repeated distillation and the sodium chloride solution by another of pure neutral zinc sulfate. In this situation no polarization was present because the electrodes were built of the same material that entered in solution. If the zinc were not absolutely pure, then the needle of the galvanometer would show a permanent deviation, instead of a null one. Regnauld solved this problem by inserting in the circuit another thermocouple having one end at 0 °C and the other at an appropriate temperature to cancel the weak current originated by the impurity. Regnauld reported that under these improved conditions the maximum electromotive forces of the gastrocnemius muscle of a frog and the trapezius muscle of the human thigh were between 4 and 5 and 9 and 10 thermoelectric units (using as unit standard the bismuth-copper couple mentioned above), respectively.36

A later publication reported the use of the method of opposition to determine the action of a current upon the mixed nerves of a frog.8

**Amalgams**

In 1826, Humphry Davy (1778-1829) discovered that amalgamated zinc was more electropositive than zinc metal.37 Many scientists had verified this fact afterwards but none had explained it in a satisfactory manner.9 Regnauld proposed the following theory: The phenomena observed in a battery composed of one pole made of amalgamated zinc and another of zinc pure depend on the difference between two actions of opposite sign, which occur simultaneously at the time of amalgamation. On the one hand, a gain of heat or of positive affinity during the liquefaction of zinc, and on the other hand, the loss of heat or positive affinity caused by its
combination with mercury. Amalgamated zinc is more positive than pure zinc because the amount of heat required to liquefy it is larger than the heat lost during the amalgamation process.

The situation with cadmium was different; the amalgamated metal, in strips, or as pasty or liquid amalgam, is always more electronegative than the metal in a solid free state. Although it seemed strange that an element so close to zinc showed a completely opposite behavior, this fact was entirely concordant with the theory described above. The thermochemical module of zinc and cadmium, relative to mercury, were almost identical but the two metals showed a very large difference in their heat of fusion, 28.13 and 13.66 cal/g, respectively. Hence, zinc became more electropositive because it gained more energy during its fusion than the energy it lost during amalgamation; cadmium became more electronegative because its heat of amalgamation was larger that its heat of fusion.9

The behavior of iron, with a heat of fusion of 64.84 cal/g, was very similar to the one of zinc; the amalgam was more electropositive than the pure metal. The same result was observed with soft iron and steel.9

In a following publication Regnauld extended his studies to a group of metals having amalgams more electropositive than the pure metal: iron, nickel, cobalt, zinc, tin, antimony, copper, lead, and bismuth. Of these, only zinc, tin, and lead combined with mercury by simple contact and without auxiliary chemical or physical action. Regnauld observed that the amalgamation of tin and lead was accompanied by a notable decrease in temperature and the positive affinity of the metal increased whenever the heat of formation of the amalgam increased. Once again, these results confirmed his the theory, as shown by the following table.38

<table>
<thead>
<tr>
<th>Metal</th>
<th>Latent heat, cal/g</th>
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<tbody>
<tr>
<td>Fe</td>
<td>64.171</td>
</tr>
<tr>
<td>Zn</td>
<td>28.130</td>
</tr>
<tr>
<td>Cu</td>
<td>33.881</td>
</tr>
<tr>
<td>Ni</td>
<td>55.397</td>
</tr>
<tr>
<td>Sn</td>
<td>14.252</td>
</tr>
<tr>
<td>Pb</td>
<td>5.369</td>
</tr>
<tr>
<td>Co</td>
<td>51.633</td>
</tr>
<tr>
<td>Sb</td>
<td>12.455</td>
</tr>
<tr>
<td>Bi</td>
<td>12.640</td>
</tr>
</tbody>
</table>

The affinity of the first three metals is slightly different from that of zinc but their latent heat of fusion is substantially higher leading to an increase in the positive affinity of their amalgams. The same argument can be used to explain the behavior of the five metals located below zinc: the heat of fusion is lower than that of zinc and combine with mercury with a such a low affinity that the process is accompanied by a decrease in temperature.38

Regnauld generalized his results as follows: (1) amalgamation of a metal changes its position in the scale of affinities. The result may be in the contrary direction even for allied metals, for it depends both on the chemical function of the metal and on its latent heat of fusion; (2) If the temperature is lowered during the combination of the metal with mercury and if, consequently, the constitutional heat of the amalgam is greater than that of the metal, the latter is raised in the order of positive affinities; and (3) When all the thermic phenomena are inverse, when heat is disengaged during the formation of the alloy, the amalgamated metal is negative, in relation to the free metal.38

It was known that thallium combined directly with mercury without the need of a physical or chemical agent. The formation of the amalgam was characterized by the change in the characteristic aspect of thallium and its separation by liquefaction in the corresponding proportion. Regnauld carried a series of experiments on the process and found that the amalgamation process was exothermic and the resulting amalgam electronegative with respect to the metal. Not only that, the position of thallium was found to be remarkably lower than that which separated cadmium from zinc in the Daniell couple.39
Alkaloids

Physical properties of quinine

In the previous years many chemists had occupied themselves in measuring the solubility of quinine salts in solvents such as water, alcohol, chloroform, and ether, and in the particular problem of substituting quinine sulfate by a derivative more soluble in water and more appropriate for hypodermic application. Regnauld believed that the information reported was very discordant, and for this reason he decided to conduct his own measurements.40 His first step was to prepare highly pure quinine by separating the raw sample from all the accompanying alkaloids. His product was anhydrous and appeared as vitreous amorphous lamellae, colorless and transparent. The alkaloid was ground finely in a glass mortar and then mixed and agitated for two hours at 15 °C with a large excess of distilled water, previously boiled. The resulting solution was evaporated and the residue dried at 110 °C. The results of three experiments conducted at 15 °C indicated that 100 g of the saturated solution contained 0.0501, 0.0480, and 0.0503 g of quinine, respectively, giving an average value of 0.0494 g, and a solubility coefficient of 1/2024, that is, 1 g of quinine per a little more that 2 liters of water. The solubility increased with temperature: at 100 °C it was 0.1314 g of quinine per 100 g of a saturated solution, corresponding to a solubility coefficient of 1/760. These figures indicated that a saturated aqueous solution of quinine, cooled from 100 °C to 15 °C, deposited about 2/3 of the solute.40

Additional experiments with alcohol, chloroform, and ether, gave the following results:

<table>
<thead>
<tr>
<th>Solubility in g/100 g</th>
<th>Solubility coefficient of saturated solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>46.866</td>
</tr>
<tr>
<td>Chloroform</td>
<td>34.177</td>
</tr>
<tr>
<td>Ether</td>
<td>4,2314</td>
</tr>
</tbody>
</table>

The exact determinations of the solubility in water permitted Regnauld to study some of the properties of quinine, dissolved in water in the ratio 1/2000, and reach the following conclusions: (1) The solubility of quinine tannate in water was less than 1/20,000 at 15 °C; (2) the fluorescent power of pure quinine increased by 25 times under the influence of an excess of sulfuric acid; (3) this increase in fluorescence allowed detecting the presence of the alkaloid in a solution containing as little as 1 part of quinine per 500,000 parts of solvent.40

Quinine tannate

In another publication Regnauld reported the results of his pharmacologic study of quinine tannate,11 a substance Pierre-Joseph Pelletier (1788-1842) and Joseph Bienaimé Caventou (1795-1877) had prepared and described during their work about quinine.41 In 1850, Charles Barreswil (1817-1870) prepared quinine tannate by adding a solution of tannin to another of quinine acetate. The resulting precipitate was filtrated, washed and dried, yielding a yellow white amorphous powder, slightly bitter and slightly soluble in water. Quinine tannate could be stored for a long time without alteration, not being affected by humidity or light. After Barreswil had suggested using quinine tannate as a substitute of quinine sulfate, physicians began administering it in the form of pills, compounded as a syrup with the pulp of a fruit, mixed with sugar and coffee, etc. A commission appointed by the Académie des Sciences reported that quinine tannate had a benign action on the stomach and intestine, did not provoke diarrhea, and its therapeutic effects were quite similar to those of quinine sulfate.42 Regnauld thought that the report to the Académie suggested it would be of interest to determine experimentally the actual process of absorption and elimination of quinine tannate, its solubility, and the possibility it could be put to more uses than quinine sulfate.11 As a result of his
experiences, Regnauld developed the following process for preparing quinine tannate, of the same composition, in a repeatable manner: A neutral solution of pure quinine acetate is slowly mixed with a solution of nutgall tannin, free of resinous matter. The resulting white jelly substance is treated with more nutgall tannin until it dissolves completely. The excess tannic acid present in the ensuing liquid is carefully neutralized with sodium bicarbonate, diluted ammonia or ammonium carbonate, and the precipitated quinine tannate is separated by filtration, left to dry in the air, pulverized, then washed with distilled water and dried again. The resulting material has an invariable composition.

Regnauld reached the following conclusions: (1) It was not possible to prepare pure quinine tannate by the simple precipitation of quinine acetate by a solution of nutgall tannin, as recommended by many scientists; the same result was obtained when replacing quinine acetate by basic quinine sulfate; (2) the compound resulting from the reaction of tannin with basic or neutral quinine sulfate retained about 3% of sulfuric acid, which could be eliminated by water washes. It constituted a kind of sulfotannic lac with properties and composition different from quinine tannate. The same sulfotannic lac was obtained when quinine sulfate was precipitated with nutgall tannin, in the presence of acetic acid. In this situation the wash water retained about 1/5 of the total quinine. The latter could be recovered by neutralization of the wash liquor with sodium bicarbonate; (3) the formulation suggested by the pharmacopeia of Strasbourg should not be used because it indicated a quinine dose almost double to the one obtained when the tannate was prepared via a double decomposition; (4) the procedure described in this memoir would always produce the same drug, containing 20.6% quinine by weight and corresponding to the formula \( \text{C}_{40}\text{H}_{24}\text{N}_{2}\text{O}_{4} \cdot 2(\text{C}_{54}\text{H}_{22}\text{O}_{34}) \), in which the tribasic tannic acid was in excess over quinine, a diacid alkaloid; (5) it had not been possible to determine the solubility coefficient of the above compound because in the presence of water it slowly split into gallotannic acid, which dissolved a small portion of the tannate, and the resulting more basic tannate; and (6) when carrying physiological and therapeutic experiences notice should be taken of the fact that one gram of neutral quinine sulfate was equivalent to 3.5 g of dry quinine tannate.11

Atropine

According to Regnauld and Valmont all the parts of the belladonna plant (\textit{Atropa belladonna}) contained atropine in different proportions, and was easily separated from its toxic derivatives. Medical atropine was general composed of two isomeric crystallizable alkaloids, which Albert Ladenburg (1842-1911) had named \textit{atropine} and \textit{hyoscyamine}, and Regnauld and Valmont suggested naming \textit{atropine} \textit{a} and \textit{atropine} \textit{b} or \textit{atropidine} (by analogy with quinine and quinidine). The properties of these two isomers were so similar that the crystalline base extracted from belladonna was prescribed and employed under the unequivocal denomination of atropine. Regnauld and Valmont added that both isomers had the same physiological, pharmaco-therapic, and toxic properties, the same influence upon the nervous centers, secreting strings of the eardrum, eye nerves (dilatation of the pupil), etc. Both isomers yielded common neutral solutions producing, for example, a white precipitate with the double iodide of potassium and mercury, a yellow precipitate with picric acid, etc. Atropine \textit{a} melted at 114 °C and atropine \textit{b} at 108 °C-109 °C. The sulfate of both isomers gave a well-defined salt, crystallizing from boiling saturated absolute alcohol and toluene, melting at 184 °C, and having a large solubility in water (about 4 parts of salt in one of water).16,17

Regnauld and Valmont summarized their findings as follows: (1) the medical atropine is a mixture in variable proportions of two isomeric crystalline alkaloids possessing the same therapeutic properties. One of them is \textit{atropine} \textit{a} (the atropine of Ladenburg) and the other, \textit{atropine} \textit{b} or better, \textit{atropidine} (the hyoscyamine of Ladenburg); (2) atropidine is present in such abundance on belladonna that it constitutes about 2/3 of the crystalline atropine defined in the Codex; (3) atropidine is the common crystallizable alkaloid present in all the mydriatic
Solanaceae and in Duboisia, and has been improperly called daturine, hyoscyamine, and
duboaine; (4) medicines containing atropidine are preferably prepared using mydriatic
Solanaceae, which contain a much higher amount of the alkaloid than Datura, Jusquiame, and
Duboisia.\textsuperscript{16,17}

**Miscellaneous**

**Amniotic fluid**

Regnauld remarked that the differences reported in the analysis of amniotic fluid arose from the
difficult in obtaining a pure sample and on the very small amounts of solids it contained, about
0.012\% of the total. In a first paper on the subject he reported the proper procedure to carry on a
qualitative analysis of this fluid.\textsuperscript{44} A very pure sample obtained from the obstetric clinic of P.
Dubois, was first evaporated under vacuum, in the presence of sulfuric acid, until it was reduced
to one-third of the original volume. These precautions were necessary to avoid the destruction of
the urea, which took place at about 90 °C to 100 °C in the presence of basic salts in the liquid. The
residue was cooled and then mixed with four or five times its weight of absolute alcohol. The
alcohol dissolved the urea and a small amount of sodium lactate and precipitated the albuminous
matter together the different inorganic salts present (e.g. sodium and calcium phosphates, sodium
chloride and sodium carbonate). The alcoholic solution was evaporated to a resinous mass and
then extracted with boiling ether, which dissolved the urea and left the lactate behind. Evaporation
of the ethereal solution left a solid composed of white prismatic needles, which proved to be urea
by different chemical and physical tests.\textsuperscript{44}

**Examination of a pathological serum**

In 1883, Regnauld and Eugène Gabriel Villejean (1850-1930) reported the composition and
properties of a suppuration extracted by the surgeon Aristide Auguste Stanislas Verneuil (1823-
1895) from an inguinal-abdominal tumor.\textsuperscript{17} This opalescent liquid had the consistency of oil, a
density of 1.038 at 15 °C, and was very alkaline; its opalescence was due to the presence of solid
particles, easily separated by filtration. An unusual characteristic of this liquid was the sudden
modification of its aspect when mixed with distilled water: from complete transparency to total
opalescence, suggesting that it mixed with water without dissolving or it separated a part (or all)
of its solutes as a precipitate. In other words, a simple contact with water led to total coagulation.
Upon mixing, the liquid recovered its transparency and the precipitate contracted to a flake having
a very small volume. The unusual properties of this suppuration led Regnauld and Villejean to try
to determine the possible conditions upon which these changes were possible.\textsuperscript{17}

Addition of a few drops of water on the surface of several cubic centimeters of the filtrated
liquid resulted in the formation of an opalescent gel of such a consistency that the tube could be
inverted without material flowing out. The same phenomenon did not take place when the
transparent liquid was added drop-wise to a few cubic centimeters of distilled water: the coagulant
action of water occurred only on the surface of the drops, forming a sort of membrane which
prevented total coagulation, even under agitation. Microscopic examination of the superficial
matter showed that it was very similar to blood or lymph fibrin. Chemical examination showed
the following results: (1) treatment of water peroxide of different concentration led to the release
of abundant bubbles of oxygen; (2) the coagulum, free of all its soluble principles, was
completely soluble in an aqueous solution of potassium nitrate (6 % wt.). This solution was
precipitated by acetic acid; (3) treatment with concentrated acetic acid or diluted HCl resulted in
swelling by hydration; (4) digestion with concentrated HCl produced a violet solution; and (5) the
coagulum was completely soluble in an aqueous solution of NaCl (7 % wt.), the same as venous
fibrin. All the above characteristics suggested that the coagulum was made of fibrin, nevertheless,
the pathological liquid being examined did not coagulate spontaneously and did not ferment even
after several days on contact with air.\textsuperscript{17}
Chemical analysis of the suppuration liquid indicated it contained, by weight, 1.86 % of fibrin, 6.24 % of metalbumin, 2.46 % of serine, 0.993 % of peptones and unidentified organic material, 0.542 % of sodium and potassium chlorides, 0.203 % of inorganic carbonates and phosphates, and 87.702 % of water. These results indicated the fibrin content was five to six times larger than the fibrin in plasma, lymph, and other known pathological products, and that the fluid examined was exempt of mucin and globulin. Regnauld worked on a variety of other subjects, among them: electrochemical properties of magnesium and gallium, a method for determining the purity of diethyl ether, determination of the solubility of ether in sugar syrups, Graham's telephone, determination of the purity of chloroform by means of aniline derivatives, the use of chloroform as an anesthetic, the presence of phosgene in chloroform, the conservation of chloroform, pharmacology of methylene chloride, methylene chloride and chloroform, and the purification of methanol.

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