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Early evolution of chromatography: the activities of Charles Dhéré☆

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ABSTRACT

Charles Dhéré (1876–1955), professor at the University of Fribourg (Switzerland), was the first in Europe to recognize the importance of chromatography. While with his students W. Rogowski and G. Vegezzi he improved the laboratory set-up used for chromatography, he also proved the correctness of Tswett's assumptions on the existence of a multitude of chromophyllic and carotenoidic pigments, demonstrated that chromatography can provide purer substances than any of the then accepted methods and further extended the use of chromatography into animal biochemistry. He also provided the first reliable discussion on the life and activities of Tswett, the inventor of chromatography.

CONTENTS

1. INTRODUCTION

In 1991 we are celebrating the eighty-fifth anniversary of the two classical publications of M. S. Tswett on the chromatographic separation method [1,2]. This development already started in St. Petersburg where he moved in 1896 and was finished in Warsaw☆☆. In the 85 years which have passed

☆ Parts of this paper were presented at the *15th International Symposium on Column Chromatography, Basel, June 3–7, 1991* and at the *8th Danube Symposium on Chromatography, Warsaw, September 2-6, 1991.*

☆☆ Mikhail Semenovich Tswett was born in Asti, Italy, in 1872. Although his father was a Russian, he spent the first 24 years of his life in Switzerland where he studied at the University of Geneva, receiving his *Docteur ès Sciences* degree in botany in 1896. At that time he followed his father to Russia. However, the Russian educational system and science establishment did not recognize foreign degrees: therefore, he had to submit new master's and doctor's theses to Russian universities. He worked on the first in St. Petersburg, in the laboratories of the Imperial Academy of Sciences and submitted it in 1901 to the University of Kazan. He received his Russian doctor degree later, in 1910, in Warsaw, where he had been associated with various universities between December 1901 and the Summer of 1915. For details on the life and activities of Tswett see the publications of Sakodynskii (refs. 3–6), Senchenkova (ref. 7) and Hais (ref. 8), and the paper of Dhéré discussed in the present publication (ref. 9).

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since these publications, chromatography became the most important laboratory method used in chemistry and biochemistry.

Tswett's invention, chromatography, and his results in the field of plant pigments, did not receive the recognition they deserved: they were greeted with skepticism and even by ridiculing them [10,11]. This so-called dormant period finally ended in 1931, when the group of Richard Kuhn, in Heidelberg, demonstrated the superiority of chromatography [12].

In the 25 years which have passed between Tswett's two fundamental papers and the start of the work in Heidelberg, only a very few scientists recognized the importance of chromatography as a separation technique and utilized it in their research. Among these two are particularly important: Charles Dhéré, in Switzerland, and Leroy Sheldon Palmer, in the USA. The purpose of this paper is to discuss in detail the activities of Dhéré and his students with special emphasis on their contribution to the evolution of chromatography. We plan to deal with the activities of Palmer in a separate publication.

2. DHÉRÉ'S LIFE; HIS FIELD OF INTEREST

Charles Dhéré (Fig. 1) was born on March 5, 1876, in Paris, and first studied medicine, receiving his *Docteur en Médecine* degree in 1898 with a thesis dealing with the variation of nerve centers as a function of size [13]. However, he never practiced as a physician. First he became an assistant at the Sorbonne, in the Department of Natural Sciences; then, in 1900, he joined the University of Fribourg, in Switzerland, as an associate professor (*professeur extraordinaire*) of physiology, biological chemistry and microbiology. In 1908, he became a full professor and, in 1909, he received the *Docteur ès Sciences* degree from the Sorbonne, with a thesis on the investigation of albuminoides, proteides and their derivatives using ultraviolet spectroscopy [14]. During his long tenure at the University of Fribourg, Dhéré served twice (in 1916–1917 and 1933–1934) as the dean of the Faculty of Science. He retired in 1938 when the title of a *professeur honoraire* was bestowed on him.

After his retirement Dhéré moved from Fribourg to Geneva where an office and laboratory space was provided for him in the Institute of Zoology of the University. He continued his scientific studies until 1951. He died on January 18, 1955[a].

Fig. 1. Charles Dhéré, in the 1930s.

Dhéré's main interest was the investigation of biologically important substances, particularly by ultraviolet and fluorescence spectroscopy. He even coined two special terms to express his field of interest: *optochimie and optochimie biologique*. As a summary of his life's activities Dhéré in 1937 published a major book on fluorescence in biochemistry

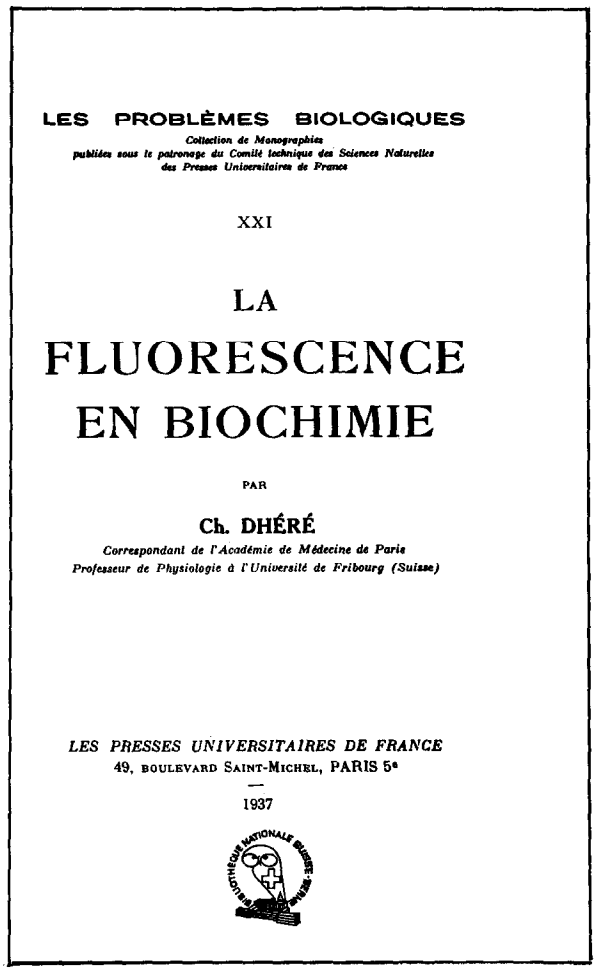
[a] Most data on Dhéré's life were taken from the eulogy by L. Laszt (ref. 15). Note that this Bulletin was always published in one volume during the summer of the following year. This is the reason that although Dhéré died in January 1955, his eulogy was published in the 1954 volume.

[16][a] (Fig. 2), which was considered at that time so important that in 1938, he received the Marcel Benoist Prize for it[b].

Naturally a prerequisite of any spectroscopic investigation is the ability to prepare pure substances. For this reason Dhéré became interested already at the beginning of his professional career in fundamental laboratory techniques which can be utilized for this purpose. One of them was electrodialysis and Dhéré contributed significantly to the advancement of this technique. His interest in methods permitting the separation of biologically important substances and their preparation in pure form also led him to chromatography.

It is interesting that Dhéré never met Tswett, not even during Tswett's frequent visits to his friend Edouard Claparède[c], in Geneva. However, obviously, he had learned about chromatography fairly early and immediately realized its advantages for his own work: after all, he needed pure substances for the spectroscopic measurements and chromatography seemed to be the ideal method for it.

Dhéré started to use chromatography around 1911 and the first work in which this is documented is the doctorate thesis of Wladyslaw de Rogowski, a student from Warsaw. A few years later, the chromatographic technique was further improved in the work of another graduate student, Guglielmo Vegezzi. Let us deal in more detail with these two students and their work.


LES PROBLÈMES BIOLOGIQUES

Collection de Monographies
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des Presses Universitaires de France

XXI

LA
FLUORESCENCE
EN BIOCHIMIE

PAR

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49, BOULEVARD SAINT-MICHEL, PARIS 5e

1937

Fig. 2. Title page of Dhéré's book on fluorescence in biochemistry, published in 1937.

[a] It is indicative that for his book (ref. 16) Dhéré selected a motto from the book of Thudichum, *On Chemical Identification of Diseases*, published in 1868, in London: "The phenomena of fluorescence will in future yield important means of diagnosis in animal chemistry."

[b] This prize was established by a Frenchman, Marcel Benoist, to honor annually important scientific inventions which particularly help to improve human life. The winner is always selected by the Swiss Federal Department of the Interior. The prize was presented for the first time in 1919. In 1938, the Prize carried an amount of 30 000 Swiss Francs, a very large sum at that time.

[c] Edouard Claparède (1873–1940) was a physician and professor of psychology at Geneva University. He had been a student at the University when Tswett studied there and they became close friends (see ref. 8).

3. ROGOWSKI AND HIS THESIS WORK

Wladyslaw Franciszek de Rogowski[d] was born on December 3, 1886, in Warsaw, in the Russian-occupied part of Poland. His grandfather was involved in the 1831 Polish uprising against Russia and this rebellious nature was evidently inherited by

[d] Rogowski's Christian name is given in different ways in the various sources, as *Wladyslaw*, *Wladislas* and *Ladislas*. The first is the correct Polish spelling of the name while the two other forms represent an attempt to spell it in the French way. Unless we quote the source verbatim, we shall give it in the Polish form.

his grandson: in 1905, as a senior in high school, he organized a strike, most likely induced by the disturbances in Warsaw in conjunction with the 1905 Russian revolution. Therefore, he had to transfer to a private school (the Jezewski School of Commerce) in order to finish his secondary education. Subsequently he went to Switzerland, to start his university studies. Again, this was probably connected with the political events in Russian-occupied Poland where, for a period, the universities were closed to students.

We have found his police registration in the city of Bern dated October 12, 1906, indicating him as a student at the University. However, he left after one year: an entry at the police dated September 14, 1907, indicates that he departed to Russia which undoubtly means his return to Warsaw. We have information that he also studied at the Jagellonian University of Cracow (in the Austrian-occupied part of Poland) which served not only the Polish people under Habsburg rule: large numbers of Poles from the territories under Russian and German rule also studied there. A Polish biography [17] indicates further studies by Rogowski at the universities of München and Leipzig, but the autobiography added to his doctorate thesis (see below) does not mention any of these.

The next definite information we have about Rogowski is an entry in the student registration book of the University of Fribourg dated October 19, 1911 (Fig. 3). He carried out his thesis work under Professor Dhéré and received his doctorate on December 21, 1912[a].

Toward the end of Rogowski's laboratory investigations a co-authored paper discussing part of his results was submitted by Dhéré to the journal of the French Academy of Sciences (where most of his papers were published): this report was presented at the October 7, 1912, session of the Academy [18]. In this short paper chromatography is indicated in a single sentence only. However, in the thesis of Rogowski [19] the technique and the system used by him is described in more detail.

In Rogowski's chromatography system (Fig. 4) the glass column was not tapered at its lower end (as originally proposed by Tswett [2]) but closed by a cork having multiple perforations; it was standing on a perforated porcelain disk held by a tapered outer glass sleeve. Calcium carbonate which was prebaked at 150°C for 10 h was used as the adsorbent; the height of the chromatographic packing in the column tube was 60–80 mm.

[a] In his later paper on Tswett and on the evolution of chromatography (ref. 9) Dhéré mentions that "my student and collaborator W. de Rogowski left my laboratory (and, most likely, also Switzerland) immediately after finishing his doctorate examination at Fribourg, in 1912." As discussed later, Rogowski's thesis was never printed and is not deposited at the Swiss National Library. We should also mention that his name is not included in the Annual Reports of the University of Fribourg which list all the persons who received a doctorate: he is missing in the Report of 1912 as well as in the Reports for the following years.

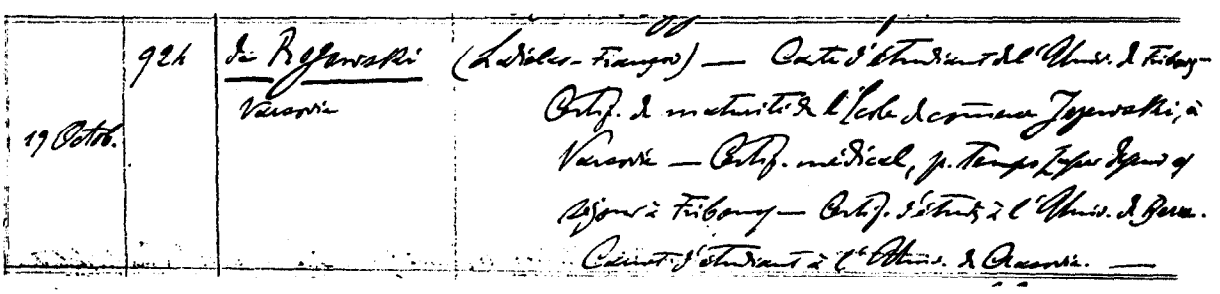

Fig. 3. Entry in the Student Registration Book of the University of Fribourg, for the 1911–1912 Winter Semester. We give the transcript of the hand-written text: de Rogowski, Varsovie, (Ladislas – Franzen) – Carte d'étudiant de l'Univ(ersité) de Fribourg – Certif(icat) de maturité de l'Ecole de commerce Jezewski, à Varsovie – Certif(icat) médical, p. temps.. (?).. séjour à Fribourg – Certif(icat) d'étud(iant) à l'Univ(ersité) de Berne. Carnet d'étudiant à l'Univ(ersité) de Cracovie.

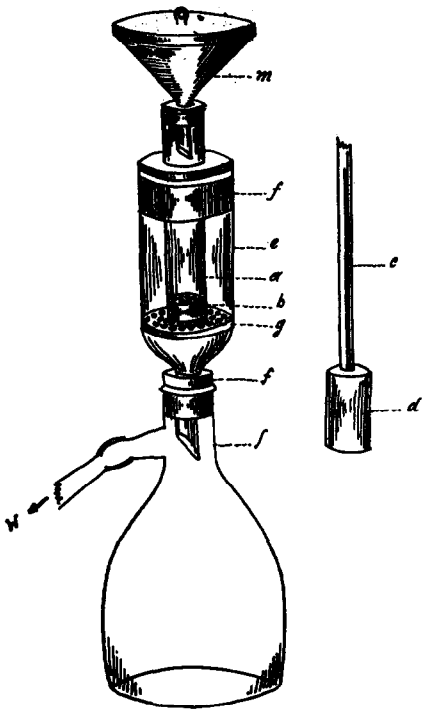

Fig. 4. Rogowski's chromatography system (from ref. 19). *a* = Chromatographic column (250 mm × 20 mm I.D. glass tube); *b* = cork (10 mm high) with multiple perforations; *c*, *d* = wooden pestle; *e* = outer glass sleeve; *f* = rubber stoppers; *g* = perforated porcelain disk; *l* = glass flask; *m* = funnel; *w* = water pump.

After separation and formation of the colored rings, the column packing (the "chromatogram") was slowly pushed out of the tube and the colored rings were carefully separated from each other and from the rest of the packing. This represented a major improvement: for a long time it had always been a problem how to isolate and collect the separated colored rings, without loss and contamination by compounds present in the other rings. Let us not forget that elution of the separated compounds from the column by a continuous solvent flow (obtaining a "liquid chromatogram", an expression used at that time to distinguish it from the column serving as the "chromatogram") started to be used only in the second half of the 1930s [11]. In his later paper on the evolution of chromatography [9] (discussed below) Dhéré emphasized this special feature of Rogowski's system, that it permits one to obtain the individual fractions without rapture of the column packing.

The subject of Rogowski's thesis was the spectroscopic investigation of chlorophyll *a* (α) and *b* (β)[a], of the so-called "crystallizable chlorophyll"[b] and of certain carotenoids.

With regard to the so-called 'crystallizable chlorophyll" Rogowski's investigations proved again that Tswett had been correct: it was not a pure substance but rather a mixture. Concerning the leaf carotenoids investigated by Rogowski, Dhéré emphasized later [9] that this work was the first after Tswett preparing pure substances and demonstrating (independently of Tswett) that pure xanthophyll does not have a red fluorescence in alcoholic solution, as described by Escher – a close collaborator of Willstätter – in his Ph. D. thesis of 1909 [23]. As stated by Dhéré [9], "if Escher had used purification by the chromatographic method, he would not have committed this error."

The main subject of Rogowski's work was the UV adsorption of chlorophyll *a* (α) and *b* (β), and an important point in the investigation was to check the purity of the isolated fractions, comparing data of Tswett *vs.* Willstätter and his collaborators. Twenty-five years later Oscar Biermacher[c] (another student of Dhéré), in his thesis [24] summarized the situation in the following way:

> "By the classical Willstätter and Stoll method of 1913, fractionation between immiscible solvents was employed to separate chlorophyll components *a* and *b* from the yellow pigments and from each other. The spectrograms inserted in the thesis of W. de Rogowski show, however, that the chlorophyll *b* previously prepared by Dhéré and de Rogowski by the chromatographic adsorption method was notably more pure than

[a] Chlorophyll *a* and *b* are identical to chlorophyll α and β; Tswett used Greek letters while later literature adapted the use of Roman characters (*cf.* ref. 10).

[b] "Crystallizable" chlorophyll was an isolated substance which Willstätter believed to be a single, native pigment. Tswett, in a number of publications (refs. 20–22) demonstrated that, in fact, is was an artifact, formed during the long alcoholic extraction of the living tissue and that it was a mixture of two substances.

[c] Oscar Biermacher was born on September 10, 1904, in Cleveland, Ohio (USA). He received the B.Sc. degree from the University of Dayton, Ohio, in 1928. After four years' teaching at high schools in Ohio, he returned to the University of Dayton in 1932 for graduate studies. In 1934 he enrolled at the University of Fribourg and carried out his thesis work under Prof. Dhéré, receiving his doctorate in 1936.

the chlorophyll *b* prepared at the same period by Willstätter and Stoll."

The same fact was emphasized in 1940 by Hans Fischer and Adolf Stern [25] who stated that

"M. Tswett was able to establish exactly the existence of two green components with help of the adsorption analysis introduced by him and to describe in detail the spectra of these pigments. These investigations were, however, later violently contradicted and they were forgotten, although, as we know it today, M. Tswett was the first who actually obtained – be it in solution – really pure chlorophyll. Only Ch. Dhéré and W. de Rogowski (1912) could again prepare pure chlorophyll solutions according to the method of M. Tswett and describe the fluorescent spectra of both components."

Although this is not the place to discuss and value Rogowski's spectra it is interesting to compare his VIS spectra of chlorophyll *a* and *b* with the ones published by Willstätter in those years. Twenty years later Alfred Winterstein [26,27] analyzed in detail Willstätter's spectra and proved that his chlorophyll *b* was not pure but had a 15% impurity of chlorophyll *a*. This is evident from the spectra published by Willstätter and co-workers [28,29] where the 644 nm absorption band of chlorophyll *b* is accompanied by a weak band at 663 nm (being the most prominent but not the only extra band in the spectrum): the 663 nm absorption is caused by chlorophyll *a* present in the solution (see Fig. 5). Today it is funny to read that Rogowski, in his thesis, was worrying about this missing band and that he only obtained *une légère ombre* (a weak shadow) at this wavelength when he increased the optical pathlength to 26 nm [19]. Obviously the chlorophylls prepared by him were really pure (Fig. 6) and indeed he confirmed Tswett's results. However, in those days Willstätter was the big authority in this field and people believed him more than the little-known Russian botanist.

It is noteworthy that F. M. Schertz (one of the translators of the chlorophyll book by Willstätter and Stoll [28]) published a paper [30] as late as 1929 in which he highly criticized Tswett's work and methodology. The following two sentences are quoted from this paper:

"It is evident that Tswett was never at any time dealing with pure pigments for not once were the substances crystallized, nor did he report any attempt made at crystallization."
"... Tswett's methods have been shown to be unreliable in identifying and distinguishing carotin and xanthophyll."

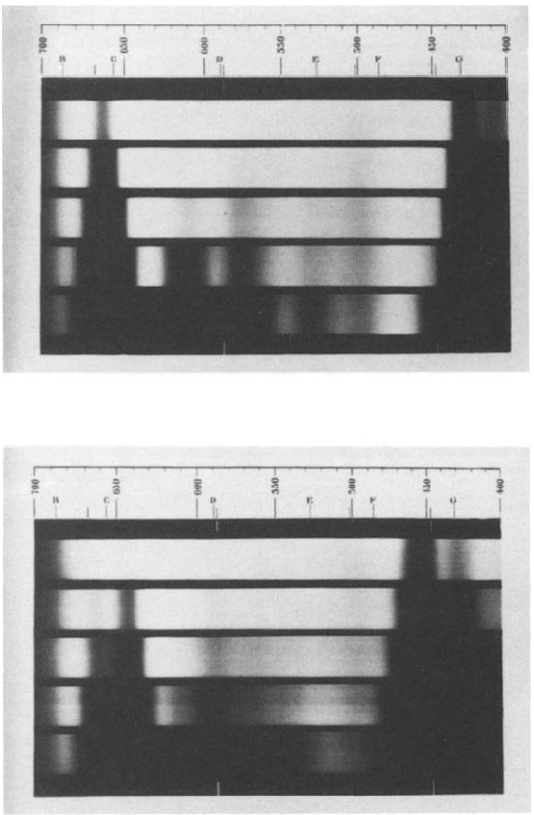

Fig. 5. Willstätter's visible spectra of chlorophylls (from ref. 28). The spectra were obtained by the direct exposure of a cell filled with solution to a photographic plate. Optical pathlengths were, from top down: 5, 20, 40 and 80 mm. Concentration: 31.2 mg of chlorophyll in 1 l of ether. The wavelength scale is in nm. Upper spectrum: chlorophyll *a*; lower spectrum: chlorophyll *b*. The 644 nm absorption band of chlorophyll *b* is accompanied by the 663 nm band of chlorophyll *a*.

It took ten more years until this argument was finally confuted by P. Karrer who stated [31] that "... it would be a mistake to believe that a preparation purified by crystallization should be purer than one obtained from chromatographic analysis. In all recent investigations chromatographic purification widely surpassed that of crystallization."

These facts emphasize even more the farsightedness of Dhéré who realized at such an early stage of the evolution of science that chromatography can give purer compounds than the classical methods.

When we were looking for the thesis of Rogowski, we could not find it at the Swiss National Library where all doctorate theses submitted to Swiss

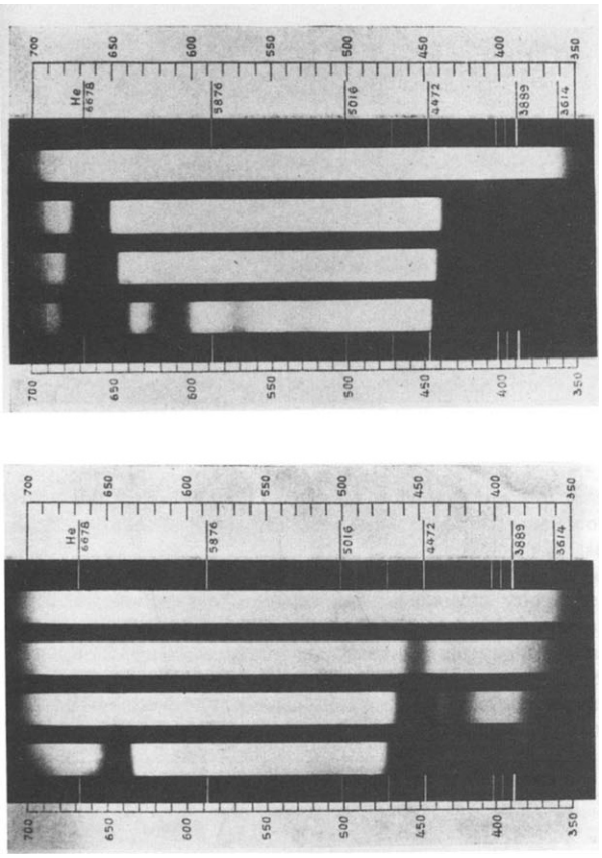

Fig. 6. Rogowski's visible spectra of chlorophylls, (from ref. 19). The uppermost strip is a blank followed by optical pathlengths of 5, 10 and 20 mm. Concentration of the solution was not known. The wavelength scale is in nm. Upper spectrum: chlorophyll *a*; lower spectrum: chlorophyll *b*. These spectra were expanded for this reproduction in order to obtain equal distances in the 400–700 nm region for Figs. 5 and 6.

universities are deposited, and there was no record of it there. Finally, we found a poor copy of the typewritten text at the University of Fribourg [19]; this text is unusual in a number of aspects. Although the text on the title page of the typed thesis specifies that it is presented by Wladislas de Rogowski to the Faculty of Science of Fribourg University in order to obtain the degree of a *Docteur ès Sciences*, the date (year) on this title page is given as 1914 (and not as 1912) and the city as Warsaw (and not as Fribourg). Also, in the brief autobiography of the author included with the thesis the fact that he received the doctor degree is given in past tense: "*A la suite de mes examens à la Faculté des Sciences de Fribourg j'ai obtenu le title de docteur le 21 décembre 1912.*" This would mean that this text is a later typescript and that he probably did not have a formal written thesis at the time of his final examinations[a].

The fact that the text we found at the University of Fribourg was typed later, outside the French-speaking part of Switzerland, is also indicated by our observation that the typewriter used did not have the accented vowels use in French (*e.g.*, *à*, *é*, *è*, etc.) and he had to add the accents by hand.

Zechmeister and Cholnoky, in the bibliography section of their fundamental chromatography book published in 1937 [32] list Rogowski's thesis as submitted to two universities: to Fribourg, in 1912, and to Warsaw, in 1914. Thus, an easy interpretation of the past tense, and of the year 1914, would be that this text was typed later and while a copy was sent to Dhéré, it was actually submitted by Rogowski to the University of Warsaw, for the second doctorate[b]. There is, however, a major problem in this interpretation. On the title page of the thesis, the author is listed as *Wladislas de Rogowski de Varsovie (Pologne)*. There is no possibility that the Universi-

[a] See footnote *a* on p. 6.

[b] In this respect we would like to refer to the well-known problem Tswett had with his Swiss doctorate. When moving to Russia, he found out that foreign scientific degrees are not accepted there; therefore, he had to repeat both his master's and doctorate degrees (*cf.* footnote ☆☆ on p. 3). It is quite possible that this "second doctorate" of Rogowski simply meant the same, submitted for official recognition.

If we assume this "second doctorate" theory, there is an intriguing possibility: in connection with submitting his thesis to the University of Warsaw Rogowski may have met Tswett. It is true that from 1908 on, Tswett was affiliated with the Polytechnique Institute and not with the University: however, undoubtedly, he maintained connections with the University where he had been active between 1901 and 1908. In his thesis Rogowski frequently cited Tswett and the thesis discussed a subject only a few could understand; therefore, it would not be unusual if his colleagues gave Tswett this thesis for review or at least, to read it. Furthermore, let us not forget that in Russia, defending a doctorate thesis had always been a well-publicized public affair. It would have been unusual if Tswett did not go to this open session: after all, the candidate used *his* method and the main subject of the thesis was to prove that *his* (Tswett's) results disputed by others were correct. The only reason which could have prevented this was Tswett's illness: we know (*cf.* refs. 5 and 8) that in 1914, he was absent between the end of March and the middle of November.

ty of Warsaw would have permitted to submit a thesis in which the country is indicated as "Poland": let us not forget that officially, "Poland" did not exist and Warsaw was in Russia[a]! On the other hand, it is also possible that this title page was prepared only for the copy mailed to Dhéré. Most likely one can never resolve this mistery.

The most interesting in Rogowski's thesis is that he quoted Tswett's magnum opus, his book published in 1910 [33] by giving its Russian title, written in Cyrillic, while *e.g.*, Vegezzi, in his thesis (see below) was quoting the book by giving its title in French[b]. We can safely assume that Dhéré already knew about Tswett's activities from his papers published in German and French scientific journals[c]. However, most likely, it was through Rogowski that he became exposed to Tswett's Russian book. Obviously Rogowski knew Russian and we would not be surprised if he brought the book with him to Fribourg. Here, we have the direct connection between Tswett's publication and the work in Dhéré's laboratory.

Just a few more words about Rogowski (Fig. 7). After his doctorate he was not engaged any more in original scientific work. He was an educator, both in Poland and Brazil (setting up schools for the children of Polish immigrants); a poet and a writer; a politician and a pioneer in modern agricultural methods; and when needed, he served his country against those who wanted to destroy it. He survived the 1944 Warsaw uprising but, on January 20, 1945, a few days after the liberation of Warsaw, Rogowski was arrested by the Red Army. He died on April 25, 1945, in the Soviet concentration camp Baskoje, in the Ural Mountains.

Fig. 7. Wladyslaw Franciszek de Rogowski, in 1935.

Rogowski's wife, Irena Maczewska, survived her husband and died on January 4, 1973, in Warsaw, at the age of 81. They had two daughters, Barbara Rogowska-Piotrowska (born in 1923, now living in Warsaw), and Kalina Rogowska-Przeslawska (born in 1927 and died in 1972)[d].

4. VEGEZZI AND HIS THESIS WORK

In 1913–1914 Dhéré had seven publications on animal and plant pigments and their spectroscopic investigations. Among these only one, the paper coauthored with Ryncki, dealing with the UV spectroscopic investigations of carotenoid pigments [35], mentioned that pure carotene was prepared according to the method of Tswett, using the technique

[a] We would like to express our appreciation to Prof. Edward Soczewinski (Department of Inorganic and Analytical Chemistry, Medical Academy, Lublin, Poland) who drew our attention to this fact.

[b] It should be noted that on the top of the front page of Tswett's book, the French translation of its title *(Les Chromophylles dans les Mondes Végétal et Animal)* is given. This can clearly be seen in the photo of the title page shown on p. 211 of Senchenkova's book (ref. 7). The identical French title of Tswett's book is cited by Vegezzi, in his thesis (ref. 34) and also in the bibliography of Dhéré's paper on Tswett (ref. 9).

[c] In the comprehensive bibliography section of her book (ref. 7) Senchenkova lists three papers in German from 1906; five in German and one in French from 1907; seven in German and one in French from 1908; none from 1909; one in German from 1910; three in German and two in French from 1911.

[d] We obtained this information and the photograph of Fig. 7 through Dr. H. Lamparczyk (Faculty of Pharmacy, Medical Academy, Gdansk, Poland) from Mrs. Barbara Rogowska-Piotrowska (Warsaw) for which we express our special gratitude.

Fig. 8. Guglielmo Vegezzi, in his middle age.

described by Rogowski, and reference is given to the paper by Dhéré and Rogowski [18].

The next major work carried out in Dhéré's laboratory in which the chromatographic technique was further improved was the thesis of Guglielmo Vegezzi, born on August 1, 1890, in Ticino, the Italian-speaking area of Switzerland (Fig. 8).

Vegezzi did his undergraduate studies at the Universities of Zürich and Fribourg; he started his thesis work under Dhéré in 1913 and it was mostly finished by the Summer of 1914. However, his military service in 1914–1915 prevented him from finishing it until the spring of 1916. He received his *Docteur ès Sciences* degree on July 25, 1916. Subsequently he joined the Swiss Federal Administration of Alcohol as a chemist, later advancing to the position of the vice-director of the Agency. He died on September 3, 1955.

The subject of Vegezzi's thesis was the spectroscopic investigation of various pigments present in invertebrates, such as in the bile and liver of the escargot *Helix pomatia*, and in the eggs of the spider crab *Maja squinado*. These investigations were very important because they represented the first application of chromatography in the preparation of such pigments in pure form, permitting the measurement of their UV absorption bands and their fluorescence. In addition to the printed thesis of Vegezzi [34] the results were summarized in six papers co-authored by Dhéré and Vegezzi [36–41].

The chromatographic system of Vegezzi (Fig. 9) differed only slightly of that of Rogowski. Now, the

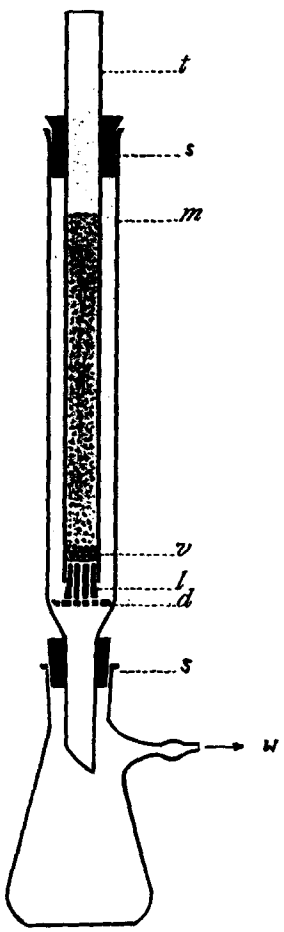

Fig. 9. The chromatographic system of Vegezzi, (from ref. 34). t = Chromatographic column (350 mm × 16 mm I.D. glass tube), m = outer glass sleeve, v = glass wool layer (about 5 mm high), l = cork with multiple perforations, d = perforated porcelain disk, s = rubber stoppers, w = water pump.

outside glass sleeve extended to the whole length of the chromatographic column: in his 1943 paper [9] Dhéré implied that in the Rogowski design (*cf.* Fig. 4), the upper part of the "chromatogram" could not be seen well because the upper rubber stopper blocked the view of it and this was the reason for the change. In Dhéré's later book on fluorescence [16] the Vegezzi design of a chromatographic system is recommended.

It is interesting to note that after finishing the investigations with Vegezzi, Dhéré evidently did not carry out any more chromatography. He also slowed down in having graduate students. In the list of Laszt [15] there is one thesis from 1906, six are from 1910–1912, one each from 1916 (Vegezzi's), 1924, 1927 and 1928, two each from 1932 and 1936 and one from 1941. In other words, there is an eight-year hiatus after Vegezzi, there are a couple of theses from the second part of the twenties and then, there is again an indication of renewed activity toward the end of Dhéré's professional career.

When going through the doctorate theses published from 1924 on, one may wonder why chromatography was no longer used in Dhéré's laboratory. A great number of pigments from various sources were prepared in pure form (or whatever was assumed to be pure) by precipitation, dialysis, crystallization and liquid–liquid partition. We may assume that the know-how for chromatography was lost when Vegezzi left Dhéré's laboratory, just after his exam, to start working in Bern. As mentioned, for some years Dhéré did not have any graduate students and the next thesis was only finished in 1924. The only one who was thinking about chromatography was Oscar Biermacher who finished his thesis in 1936 [24]. In this he discussed the method, but then decided to use liquid–liquid partition for the isolation and purification of chlorophylls, because he found that this technique is not inferior to chromatography. This is even more strange because by then, chromatography started to be widely used in many laboratories, also in Switzerland.

We have discussed the activities of Dhéré only from the point of chromatography. An investigation of his whole professional life was beyond our task.

5. PAPER ON TSWETT AND ON THE EVOLUTION OF CHROMATOGRAPHY

In 1937 Zechmeister and Cholnoky published their bestseller, their book on chromatography [32] and one year later a second, greatly enlarged edition was published. In the preface of the second edition, the authors stated that "it was intended to begin this volume with a biography of Tswett, but reliable data about the life of this pioneer were not available up to this time". Indeed this was true: the first brief biography of Tswett (a total of 28 lines, with a bibliography listing 56 publications) was published only in 1940, in a collection of the biographies of botanists from Geneva [42] written by John Briquet[a].

Evidently this publication induced Dhéré to start collecting data on Tswett. Dhéré was now retired and living in Geneva, with strong connection to the University. Unfortunately Edouard Claparède, Tswett's closest friend in Geneva, with whom he studied at the University and whom he visited almost yearly from Russia[b], was already dead when Dhéré became interested in Tswett's life and activities, but his widow provided a photo of the young Tswett which he presented in 1896 to his friend Claparède. However, Dhéré could still contact P.G. Hochreutiner, professor at Geneva University, who was also a graduate student together with Tswett in the laboratory of professor Marc Thury.

It took about two years for Dhéré to compile his 50-page long paper which he finally submitted on March 23, 1943 to the journal *Candollea* [9]. This paper not only dealt with the life of Tswett but it also discussed in detail his scientific work presenting a critical evaluation of the controversies related to the early years of chromatography and to Tswett's chromatographic investigations of plant pigments. In addition, Dhéré also presented a summary of the evolution of chromatographic analysis up to 1940. The article finished with a brief discussion of the

[a] John Briquet (1870–1931) was assistant at the Botanical Institute of the University of Geneva when Tswett studied there. He later became professor of botany at the university. After Tswett's return to Russia, they remained in touch through frequent correspondence. Briquet finished the compilation of ref. 42 just before his death but it was published only nine years later, on the occasion of the 50th anniversary of the Société Botanique Suisse.

[b] See footnote *c* on p. 5.

influence of Tswett's method on the results of "the princes of contemporary science", the Laureates of the Nobel Prize in Chemistry. Finally a detailed bibliography listing the most important papers related to chromatography, including 36 papers by Tswett, was given[a]. For 30 years, until the start of the publications of Sakodynskii, Senchenkova and others, this was the most comprehensive discussion of Tswett's life and activities.

6. CONCLUSIONS

In the early evolution of chromatography Charles Dhéré occupies a very important place. He was the first who, by independent investigations, proved the correctness of Tswett's assumptions on the existence of multitude of chlorophyllic and carotenoidic pigments. Dhéré extended the use of chromatography into animal biochemistry[b] and demonstrated that indeed, chromatography can provide purer substances than any of the then accepted methods. We might rightly cite Winterstein and Willstaedt in praising his activities: In 1933 Winterstein stated that "the significance of this method (*i.e.*, of chromatography) to biochemical research subsequently (*i.e.*, after Tswett) appeared to be understood only by Charles Dhéré and his co-workers" [26]; and, according to Willstaedt, "one of the first authors to appreciate the advantages of this new procedure (*i.e.*, chromatography) was Dhéré, who made use of it in a number of investigations" [43].

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NOTE ADDED IN PROOF

In our paper we often used the term "Russia": by this we simply referred to the Russian Empire of the Tsar prior to 1916 and not to the geographical area of Russia formed after the dissolution of the Soviet Union at the end of 1991. Similarly, the term "Russian" meant a subject of the Russian Empire.

Actually, the true nationality of M. S. Tswett and of his father is not so simple. His father, Semen N. Tswett, was born in Chernigov which is in the Ukraine; thus, according to present-day standards he might be considered a Ukrainian. However, he left that area as a teenager and never returned (except for brief visits). He studied at the University of Tartu (the same school where, 70 years later, his son became a professor) in Estonia (which, at that time, was part of the Russian Empire) and, after graduation, he had been active in Russia proper or abroad (in Italy and Switzerland). In the last few years of his life he was a Councilor of State, the Chairman of the Provincial Finance Department in the Crimean Peninsula which then was part of Russia proper but today, because of a decree of Khrushchev from the mid-1950s, belongs to Ukraine.

Mikhail S. Tswett's mother, who was of Italian origin, was born in Turkey but grew up in Russia. As mentioned (*cf.* footnote *a* on p. 4) M. S. Tswett was born in Italy and grew up in Switzerland. In fact, when he returned to Russia, in 1896, he spoke only fairly poor Russian and we know for instance (see ref. 8) that at home, he and his wife preferred to

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[a] We have found two apparent errors in Dhéré's paper: (a) the date of Tswett's death is given as May 1920, while he actually died on June 26, 1919. This error can be traced back to the announcement of Tswett's death in the *Berichte der deutschen botanischen Gesellschaft* giving this false date. (b) Dhéré stated that Edouard Claparède died in 1939; however, according to Hais (ref. 8) he died in 1940.

[b] Actually L. S. Palmer at the University of Missouri (Columbia, MO) in the USA, also carried out chromatographic investigations of pigments present in plants and animal tissues, from 1911 on. However, at that time, neither knew about the other's work.

use French in conversation. In his life M. S. Tswett spent 25 years in Switzerland, seven years in Russia proper, fifteen in Poland and less than one in Estonia (both within the Russian Empire). So what nationality was he?

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