THE DISCOVERY AND EARLY HISTORY OF CAROTENE

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"Wild carrot, Daucus carota, *a member of the natural order Umbelliferae, grows wild in fields and on roadsides and sea-shores…It is the origin of the cultivated carrot, which can be developed from it in a few generations…As usual in members of the order Umbelliferae, the wall of the fruit is penetrated lengthwise by canals containing a characteristic oil."* Encyclopedia Britannica, Eleventh ed. 1910.

Carotenoid pigments are today counted in the many hundreds. The best known member of this group is carotene, which plays a remarkable role in health and medicine as the progenitor of Vitamin A. When one examines the record, it becomes evident that carotene was discovered as a byproduct of the search for a medicinal agent of a quite different kind, namely, an anthelminthic for use in ridding the body of parasitic worms, especially from the intestine. Credit for that discovery goes to the German pharmacist, Heinrich Wilhelm Ferdinand Wackenroder.

Heinrich Wilhelm Ferdinand Wackenroder (1798-1854)

In June, 1826 Wackenroder (Fig. 1) published his doctoral dissertation, "On Anthelminthics in the Vegetable Kingdom," as presented to Göttingen University. The thesis earned him very high praise, as well as the Royal Prize (1). A few years later he published the results of his examination of carrots, one of the purposes of that research being the search for the presence in the juice of that vegetable of an effective anthelminthic (2). According to a statement in that paper, "The carrot-juice provided good service in helminthiasis." This prompted Wackenroder to undertake chemical analysis of the juice in the attempt to identify the constituent that was medically active. The results of this work were published in 1831 (3). In that paper, he described how he pressed out carrots, obtaining a significant amount of a reddish juice." In fact, the juice was brick-red in color, turbid, with an aromatic taste, which was at the same time somewhat sweet and tart. Wackenroder described how he diluted the juice with water, and then extracted the liquid with ether. Upon evaporation of the ether extract, there resulted "a yellow fatty oil and carotin" (4). His first attempts to separate these two components failed. Furthermore, the oil underwent rancidization, which consequently affected the carotin; but eventually he obtained the pigment as small ruby-red flakes. These were odorless and tasteless, and showed no reaction with litmus paper or turmeric paper. The crystals were soluble in ether, less so in absolute alcohol, and not at all in water. Dissolved in fats like butter, they imparted "a beautiful yellow color."

Wackenroder, the discoverer of what was to become known as "carotene," was born in Burgdorf, near Hannover, Germany, in 1798. His father, qualified as a physician and as an apothecary, practiced both professions. Young Wackenroder had his first professional training as an apothecary and worked in that capacity for his father for two years. In 1819 he began the study of pharmacy, medicine, and science at Göttingen University.

***Figure 1**: H. W. F. Wackenroder*

After graduation in 1825 he became assistant to Friedrich Stromeyer in the Göttingen Pharmacy Institute. Having obtained the D. Phil. degree, in short order he was promoted to Privatdozent, but was then attracted to the University of Jena as Professor extraordinarius. In 1836 he was appointed Professor ordinarius and Director of the Pharmacy Institute, later known as the "Chemical-Pharmaceutical Institute." He became especially known for his discovery of carotene and his correspondence with Goethe. In subsequent years he became co-editor of *Archiv der Pharmazie*, and was elected to the Leopoldina and to many foreign societies, a clear reflection of the eminent position to which he had risen in science (1, 5).

His research was mainly concentrated in plant chemistry, his discovery of carotene in the ether extract of carrot juice being a prime example. He is still remembered for his discovery of the solution of polythionic acids that result from the reaction of dilute sulfurous acid with hydrogen sulfide.

Wackenroder's accomplishment in characterising "carotin" stands in contrast to the efforts of two earlier outstanding French chemists, Edmé Jean Baptiste Bouillon-Lagrange (1764-1840) and Nicolas Louis Vauquelin (1763-1829). Lagrange held a senior position at the Paris School of Pharmacy and at the same time was a Health Officer of the Army of the French Republic. He later was appointed Professor of physics and chemistry at the Central School of the Pantheon and at the Polytechnical School (6). He was the author of many papers in the field of pharmaceutics. His attempts to analyze the pigment of carrot-root failed; he could describe it only as "yellow oily material."

Nicolas Louis Vauquelin, born in the north of France at St. André d'Hébertot (Calvados) in 1763, died in his native town in 1829. He came from a poor peasant family but was recognized for his brilliance and was helped to obtain a good education. When he was fourteen years old he left home, eventually arriving in Paris where, by chance, he came into contact with Fourcroy, who gave him the freedom of his laboratory. In 1792 he received his pharmacy degree. Two years later he won a post at the new School of Mines, and soon after an additional post at the École Polytechnique. In 1801 he was named Professor of Chemistry at the Collège de France and, in succession, assayer at the Mint (1802), Director of the School of Pharmacy (1803), and Professor of Chemistry at the *Jardin des Plantes* (1804) and at the Faculty of Medicine (1811) (7). Vauquelin was regarded as a leading analytical chemist in Europe (8). His examination of carrots led him to conclude that the material he was after was simply "a resinous pigment" (9).

Hydrocarbon Nature of the Carrot Pigment

Some sixteen years after Wackenroder's identification of the carrot pigment, William Christopher Zeise (1789-1847) attempted to purify it. Zeise was born in Slagelse, Denmark, a town south of Copenhagen, in 1789. His father was a pharmacist, a profession which the young Zeise eventually entered. His studies of chemistry convinced him of Lavoisier's novel teachings, which he took up enthusiastically. For a few years beginning in 1806 he served in Copenhagen as assistant to H. C. Oersted (1777-1851), but in 1809 he began studies that led to his receiving a degree in pharmacy in 1815. Two years later he was awarded the doctorate. Specializing in analytical and organic chemistry, he discovered mercaptans and thioethers (10).

It was during his appointment at the Copenhagen Polytechnic Institute that Zeise undertook his work on carotene, perhaps the last of his studies, for he died in 1847, the year that his articles on that subject were published. In the two nearly identical papers (11), Zeise described his procedure, starting with the press-juice of the root vegetable. On its acidification with dilute sulfuric acid, a colored precipitate developed, which was freed from oily matter (and some protein) by treatment with alkali. In a novel contribution to this work, Zeise found that carotene is soluble in carbon disulfide, yielding a red color. From this extract he was able to obtain small, dark red, lustrous crystals, with melting point 168°. Zeise recognized that he was dealing with a hydrocarbon, but his analyses gave him C_5H_8 as reported in one of his papers, and a ratio of the two elements of 5:10 in the other. As ß-carotene has the molecular formula $C_{40}H_{56}$, it is evident that he had not succeeded in obtaining a pure product.

Some years later August Husemann (1833-1877) took up the study of carotene. Husemann had studied pharmacy at Göttingen, receiving his D. phil. in 1860. After graduation he remained at that university as assistant in the laboratory of physiological chemistry. In 1865 he took up a position as Professor of chemistry and physics in the Cantonal School in Chur, Switzerland. His publications include texts in plant chemistry and toxicology (12). Husemann, recognizing that carotene is an unsaturated compound, prepared halogenated derivatives of it (13). He also described the gradual bleaching of the pigment upon exposure to air. The product, unlike carotene itself, was now practically insoluble in carbon disulfide and benzene but was readily soluble in alcohol and ether.

Zeise's recognition of carotene as a hydrocarbon was confirmed many years later by Albert Léon Arnaud (1853-1915), at one time a pupil of Michel Chevreul (1786-1889), and later professor of organic chemistry at the Museum of Natural History in Paris. Arnaud dealt mainly with plant chemistry, including the study of toxic substances of plant origin, such as strophanthine, digitalis, and ouabaine. His analysis of the sample of carotene he had prepared from carrot juice demonstrated the presence only of carbon and hydrogen. His calculations led him to propose the formula $C_{26}H_{38,}$ which is close to the theoretical proportions (14). He noted that his purified carotene crystallized in thin rhombic plates, exhibiting dichroism, and that it was easily oxidized and halogenated.

Among his achievements, Arnaud developed a colorimetric method for the determination of carotene (against a standard he prepared from carrot juice), which he applied to the determination of that pigment in some 30 different plants (15). In that paper Arnaud suggested that carotene plays a role in oxygen transfer reactions, somewhat analogous to the action of hemoglobin in blood. This concept was subsequently taken up by a few experimenters, including Richard Willstätter and Heinrich Escher (16), but without concrete results.

Carotenoids in Animal Tissues

The identification of carotene in animal tissues came about through the initiative of Adolf Lieben (1836-1914) (Fig. 2), a young Austrian chemist, when he took up a position at the University of Palermo in 1863. Lieben came from a very well-to-do family, whose members were occupied with various business interests, but with concern for the progress of science and the recognition of scientific achievement (17). Adolf, unlike other members of his family, chose to follow an academic career. After being home-schooled as a youth, he developed an interest in chemistry while studying at the Vienna Polytechnikum and the University of Vienna. He spent some time in the laboratory of Robert Wilhelm Bunsen (1811-1899) at the University of Heidelberg in 1855, where he met Henry

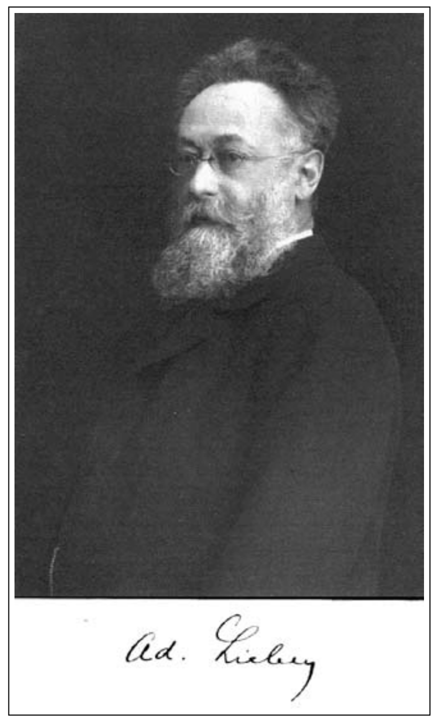

Figure 2. *Adolf Lieben*

Roscoe (1833-1915), Julius Lothar Meyer (1830-1895), and Friedrich Konrad Beilstein (1838-1906). Upon receiving his D. phil. from Heidelberg, he moved to Paris to work with Charles Adolphe Wurtz (1817-1884). Here he became acquainted with Charles Friedel (1832-1899), Alexander Butlerow (1828-1886), and Stanislao Cannizzaro (1826-1910). After a brief period as industrial chemist in Lille, he accepted an invitation tendered by Cannizzaro to join the faculty of the University of Palermo. Cannizzaro, a native of Palermo, and now professor of chemistry at its university, was seeking to build up his department. Lieben taught there for a few years (1863-1867) and then moved to the University of Turin. In 1871 he accepted a professorship in Prague. In 1875 he was appointed professor of general and pharmacological chemistry at the University of Vienna. He died in Vienna in June, 1914 (18).

During his career, Lieben published extensively in organic chemistry. The work relevant to carotenes is his study of the pigment occurring in the corpus luteum *(yellow body),* an organ that arises in the ovary of mammals immediately following ovulation. It is highly developed in some species. For example, in the cow it may occupy much of the ovary, so that its prominence must have long been recognized by butchers and those who observed their work. But the nature of the colored matter was unknown. This was one of the problems Lieben undertook during his period in Palermo.

He and his collaborator G. Piccolo began their investigation by accumulating a large number of bovine corpora lutea (19) which, after undergoing air-drying, were cut into small pieces and then extracted with ether. This yielded a yellowish solution. After evaporation of the solvent, the residue was boiled with concentrated potassium hydroxide for many hours. The nonsaponifiable (water-insoluble) fraction was found to contain two compounds: cholesterol and a red crystalline substance. Piccolo and Lieben, assuming the latter was a product of animal metabolism, sought to identify it with one of the limited number of colored substances known in animals. Hemoglobin, or rather its heme prosthetic group, seemed an obvious source. Piccolo and Lieben were able to eliminate bilirubin as a candidate and also 'hematoidin,' a presumed, but never identified, iron-free porphyrin metabolite or mixture of compounds. Concluding that the red crystalline substance they had extracted from the corpus luteum of the cow was a new derivative of hemoglobin, Piccolo and Lieben chose the alternative names 'luteo-hematoidin' or 'hemolutein.'

Another chemist, F. Holm, based in St. Petersburg, but working temporarily in Städeler's laboratory in Zürich, had begun the search for hematoidin in pathological brains of persons dying of stroke, but soon turned to the more readily available corpora lutea of cows. He mistakenly concluded that the pigment therein was hematoidin (20).

Lieben was thus the first chemist to study in animal tissue what came to be known as carotenoids. These pigments occur not only in the corpora lutea, but also in nervous tissue, retina, adipose tissue, and various viscera. It has long been known that plant foods are the source of the pigment in animal organs (21). This is demonstrated clearly by investigations showing that carotene accumulates in tissues in proportion to its availability in the diet (22). ß-Carotene has long been recognized as the precursor of vitamin A, an important dietary requirement for animals. Whether these pigments play additional roles in the animal organism is being investigated in some laboratories and clinics. For example, recent studies suggest some role for ß-carotene in luteal function, at least in the cow (23).

The 'Luteines' of J. L. W. Thudichum (1829-1901)

At about the same time that the Palermo workers were isolating the luteal pigment, Johann Ludwig Wilhelm Thudichum (Fig. 3) was conducting research on the "yellow organic substances contained in animals and plants." Thudichum was a physician who, after graduating from the University of Giessen, received training in chemistry under Justus Liebig (1803-1873). On emigrating to London, Thudichum established his medical practice, but for a time he also taught at a now defunct medical school. His enthusiasm for the application of chemistry to medicine caught the attention of John (later, Sir John) Simon, the Medical Officer of Health for England and Wales. Holding similar views himself, Simon was quick to employ Thudichum as a part-time research consultant (24).

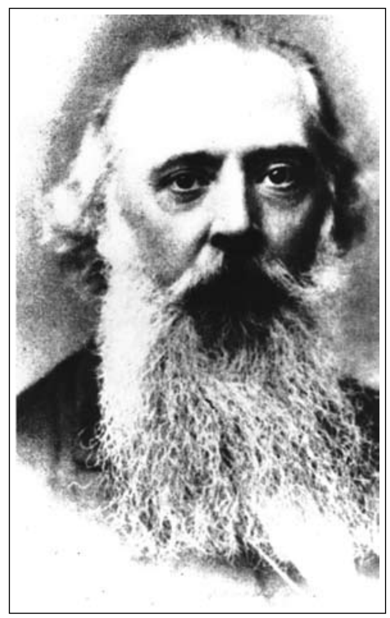

Figure 3. *J. L. W. Thudichum*

One of the techniques Thudichum used in his work was spectroscopic analysis, which he had learned in

Heidelberg from Bunsen in 1855 during an interlude in his medical studies. In 1868 he published a classic paper in the *Proceedings of the Royal Society* (25), in which he provided spectroscopic details for a multitude of colored substances, among them Wackenroder's carotene. Although Thudichum was aware of Holm's paper, but apparently not of the work of the Palermo chemists, the spectrum for carotene proved to be virtually identical to that of Piccolo and Lieben's luteal pigment (25). Thudichum assigned the name "luteine" to this yellow crystallizable substance. He pointed out that (25):

> [I]n the vegetable world it is observed in seeds, such as maize; in the husks and pulps of fruits, such as annatto; in roots, such as carrots; in leaves, such as those of the coleus; and in the stamina and petals of a great variety of flowers.

He examined 42 different plants in this study. Thudichum's luteine, insoluble in water, was easily soluble in alcohol, ether, and chloroform, forming yellow solutions. The chloroform solution when concentrated had an orange-red color. Thudichum is widely acknowledged as the first to define these yellow organic pigments as belonging to a new class of organic compounds (26).

Richard Martin Willstätter (1872-1942)

In the course of his extensive investigations into the chemistry of chlorophyll, Richard Willstätter (Fig. 4), working at the Swiss Polytechnikum in Zürich (27), also paid attention to these yellow pigments. The results of these studies appeared between 1907 and 1913. He and his assistant Walter Mieg first of all distinguished between the hydrocarbon carotene, to which they assigned the formula $C_{40}H_{56}$ (28), and the similar, but oxygen-containing, xanthophyll, having the molecular formula $C_{40}H_{56}O_2$. The two compounds were distinguished by their contrasting solubilities: carotene being easily soluble in petroleum ether, but not alcohol; and xanthophyll exhibiting opposite solubility properties. With Heinrich Escher, Willstätter identified the pigment lycopene in tomatoes. By comparing this with purified carotene as to crystal structure and other physical properties, they demonstrated that lycopene is an isomer of carotene (29).

These authors then carried out an examination of the pigment occurring in egg yolk (30). The question was whether it was carotene or some other compound. Previously Chevreul, Théodore-Nicolas Gobley (1811-1876), and Georg Städeler (1821-1871) had attempted to identify the pigment, but without success. Now, from 6,000 hen's eggs Willstätter and Mieg isolated a pigment belonging to the xanthophyll group, and to which they gave the name 'lutein.' Considering that they recognized Thudichum`s work, their choice of the same name for a different product was unfortunate. However, this class of pigments soon became known as "luteines," a name that was eventually superceded by the rubric "carotenoids." The next publication from Willstätter's Zürich laboratory dealing with this subject was by Escher, who studied the pigment of the cow's corpus luteum (31). Escher found that the addition of carbon disulfide to the yolk extract did not yield a distinct red color, given by carotene, as in the extract of corpus luteum. Moreover, the compound extracted from hen's eggs yielded crystals of a different color from those obtained from the corpus luteum. Escher`s work thus distinguished between the egg yolk and luteal pigments and established carotene as characteristic of the corpus luteum of the cow.

It is interesting that when Willstätter came to write his memoirs (32), he devoted little space to the work on the carotenoids. His main concern was clearly his studies of the chemistry of chlorophyll. This work was prominent in his being awarded the Nobel Prize in Chemistry in 1915 (33).

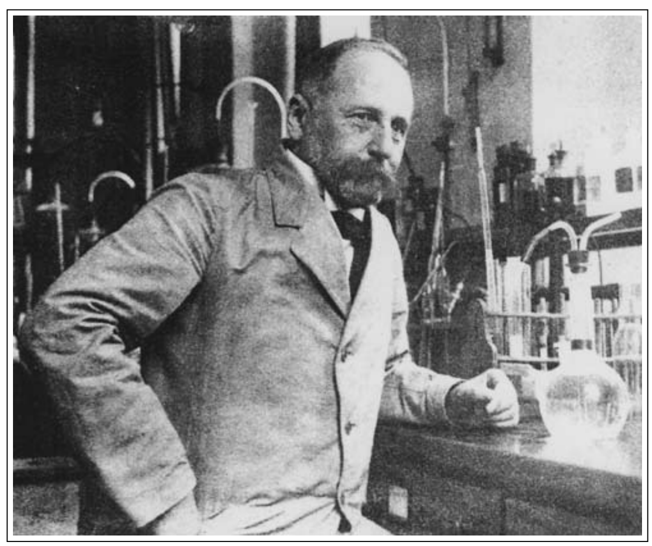

Figure 4. *Richard M. Willsätter in the laboratory*

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