THE DISCOVERY OF LECITHIN, THE FIRST PHOSPHOLIPID

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Phospholipids (1) have important functions as key elements of cell membranes. In recent years they have been recognized also as the source of important intracellular messengers, thus endowing them with more than a structural role. Their discovery at the beginning of the nineteenth century is intimately tied to the beginnings of the modern study of the chemistry of the brain. This paper traces the events leading up to the discovery of lipid-bound phosphorus (2) in the brain and some other tissues. The story unfolds through the work of six chemists, spanning more than a century, the first of whom was Johann Thomas Hensing.

Johann Thomas Hensing (1683-1726)

Hensing was born in Frankfurt/Main on August 30, 1683, into a medical family. At the age of 18 he enrolled in the Philosophical Faculty at Leipzig. His plan to study theology was interrupted by illness. Following restoration of his health, he registered as a student in the Medical Faculty. He completed his studies in Giessen where, except for a brief period in Frankfurt, he remained for the remainder of his life.

Initially Hensing was district medical officer in Giessen, but in 1712 he was appointed Privatdozent in Medicine at the University. In 1717 his status was raised to that of Professor Extraordinarius, and five years later he received the title of Professor Ordinarius of Natural and Chemical Philosophy in the Philosophical Faculty. He was fortunate in his promotions in having had Pro-

fessor G. C. Möller as his mentor when beginning his medical practice. Möller had been in charge of the teaching of chemistry in the Medical Faculty, and Hensing succeeded him in this responsibility. Laboratory facilities allowed the young professor to carry out his notable study of the chemical composition of the brain, the results of which he published in 1719 (3).

Hensing chose the brain as an object of study because he recognized that organ as "truly the throne of the soul and the abode of wisdom, from whose nature the former is the recipient of the virtues of health, and the latter of brilliance (4)." To apply chemical analysis in the effort to understand such abstract conceptions was, indeed, a materialistic proposition, although Hensing could hardly have expected to end his work with a precise chemical discovery about the brain. He published the results of his study in Latin, with the title: "The Chemical Examination of the Brain and the Unique Phosphorus from which it Ignites all Combustibles." The translation of the essay is by Tower (5).

Hensing's analysis of the brain included the 'volatiles' (chiefly water), solids, and ash. Examination of the last item revealed the presence of elemental phosphorus. This was a highly original discovery, for until Hensing's work, phosphorus had been found only in excreta, from which it was prepared commercially, and in the ash of vegetable matter. Although his finding was mentioned subsequently by a few writers, there is no reference to it in the writings of the most popular

authors of chemistry texts and compendia at the end of the eighteenth century. The work of the Giessen chemist probably received more attention only after it was mentioned by Johann Friedrich John (1782-1847) in his translation of N. L. Vauquelin's thesis (6) and in his comprehensive chemical tables of the animal kingdom (7). Many years later J. L. W. Thudichum (1829-1901) noted that Hensing's discovery was (8):

The earliest distinctly chemical fact ascertained by research conducted on brain matter. ... The discovery of [phosphorus there] was no doubt made by the methods of Brandt and Kunckel, the discoverers of phosphorus, and was one of the many results of the great impulse which the then marvellous productions of these accomplished apothecaries had given to the study of chemistry in the principal European countries.

Finding phosphorus in the brain was especially intriguing, for its properties, especially its light-emission (phosphorescence), suggested to some a kind of relationship to thought and the production of 'ideas.' Many years later the French physician and philosopher Georges Cabanis (1757-1808) proposed an intimate relationship between phosphorus and mental states, even implying that the element is

formed in the brain. Cabanis's view exerted an influence well into the nineteenth century (9), despite the disproof of phosphorus's vital origin by John (10).

Another work by Hensing bears an interesting title (11):

Dr. Johann Thomas Hensing extends a courteous and loving invitation to the senior members and patrons of the Academy, as well as to the most excellent, illustrious and honorable citizens to [attend] the funeral solemnities of Lais—not she of Greece [i.e. a famous courtesan], but rather of the whole world—that is, of alchemy, who is thought to be the elder daughter of Chemistry, which will take place on a coming day in October, and in which he will publicly conduct chemical demonstrations.

It is noteworthy that Hensing addressed his book not to some exalted sponsor, but rather to a wide audience of readers interested in science, which was now progressing beyond alchemy.

Antoine-François Fourcroy (1755-1809)

Fourcroy was born in Paris, the son of a much respected pharmacist. He studied medicine, receiving his degree in September 1780. Shortly thereafter he was elected to associate membership in the Royal Society of Medicine.

He had already chosen chemistry as his field of professional interest and was lecturing on the subject even before completing his medical course. He opened a private laboratory in which a succession of brilliant young men received their training. Fourcroy was soon appointed professor of chemistry at the Royal Veterinary School at Alfort and also at the Jardin du Roi. He was named Director of the Museum of Natural History (12).

Fourcroy investigated a large number of diverse subjects, publishing many scientific papers, often with his protégé and friend Nicolas-Louis Vauquelin (1763-1829). When Fourcroy was appointed in 1785 to the commission to oversee the removal of the

Cemetery of the Holy Innocents in Paris to the Catacombs, he and the head of that body, M.-A. Thouret (1748-1810), found the opportunity to make chemical observations on some of the cadavers. They were especially impressed with the apparent state of preservation of the brain, even in corpses long interred.

Fourcroy soon realized that to obtain reliable data, it would be necessary to work with fresh brains. It is noteworthy that he eschewed the older methods that depended so largely on distillation and capturing of the degradation products for analysis. He used extraction procedures with aqueous solvents and alcohol, methods that led him to the conclusion that the brain consists of "animal pulp," (largely protein), fatty substances that he regarded as "soaps," and salts, chiefly phosphates of calcium, ammonia, and sodium (13). Among the fatty



Johann Thomas Hensing, M.D. circa 1724 (D.B. Tower, Ref. 3)

substances was a 'greasy oil,' later to be recognized as phospholipid. Neither in his "Cemetery papers" nor in his extensive compendium of chemistry does Fourcroy make mention of Hensing's work. He was apparently unaware of the prior finding of phosphorus in the brain (14).

Johann Ludwig Jordan (1771-1853)

A decade after Fourcroy's studies of brain chemistry, the German chemist Johann Ludwig Jordan undertook to repeat his work. Unlike Fourcroy, who was attracted to brain studies through rather practical considerations, Jordan expressed his interest in the composition of the brain philosophically (15):

We must well wonder that one of the most important animal substances, in which the origin of mind and the seat of the soul have been sought, has thus far so little aroused the curiosity of chemists. Blood, bile, milk and other matters have already been worked upon so often and repeatedly that our knowledge of these is considerable, whereas we still stand almost in the dark here [i.e. with respect to the brain.] Is the brain then not less important? It would be indisputable to wish very much that chemists might agree to work upon this important subject, just as has been done for other animal substances.

Jordan was born in Göttingen on June 6, 1771. He attended the university there, eventually receiving a degree in medicine. For a short while he had a medical practice in Clausthal, but his interest in chemistry soon drew him away from medicine. He became committed to analytical work in mineralogy, and in his papers on the brain he states that he is no longer in a position to carry on the work of Thouret and Fourcroy. He ultimately was appointed Master of the Mint in Clausthal (16).

The essence of his work on the brain is as follows: the desiccated tissue, when burned in an open crucible, gives an acid reaction, which he suspected was due to phosphoric acid contaminated with sulfuric acid. An aqueous extract of brain from which the protein had been precipitated was treated with ground lime; this gave rise to ammonia, presumably released from ammonium phosphate. In another experiment he was able to isolate, with the addition of limewater, calcium phosphate.

Jordan carried out many other experiments, from which he concluded that the brain mass contains water, albumin (protein), sodium, ammonium, and calcium phosphates, and "a characteristic fatty material." This last component corresponds to Fourcroy's 'greasy oil,' and represents confirmation of the work of the French scientist. Jordan regarded his lipid extract as a distinctly animal product not encountered elsewhere. He located it in the medullary portion of the brain [*i.e.*, the white matter] and in the marrow of nerves (15).

Jordan retired in 1845 on a pension and died on May first, 1853 in Osterode, not far from Clausthal.

Nicolas-Louis Vauquelin (1763-1829)

Vauquelin has been the subject of numerous biographical articles and eulogies that describe his rise from impoverished family origins in St. André d'Héberdot, in Calvados, Normandy, to that of the élite of French science in the first three decades of the nineteenth century (17). His career began when he arrived in Paris and found work in a pharmacy, where he had the good fortune to meet Fourcroy. The senior chemist took the young man under his wing in 1784, giving him a post in his own laboratory. This was a major step in Vauquelin's professional development. Having obtained his diploma in pharmacy in 1792 and master's degree in 1795, he was invited to join the faculty of the School of Pharmacy, shortly thereafter becoming Professor of Chemistry there, and eventually its director, from the time of its reorganization in 1803 until his death in 1829. Under the French system permitting the holding of multiple posts, Vauquelin was also Professor of Chemistry at the Museum of Natural History and at the Medical Faculty, and for a time was Master of the Mint. He was celebrated throughout Europe for his achievements in analytical chemistry, as well as for his discovery of chromium and beryllium.

Following the death of Fourcroy in 1809, Vauquelin was appointed to the chair of chemistry at the Faculty of Medicine, despite the fact that he lacked a degree in medicine. His very extensive medical knowledge, together with a thesis on the subject of the analysis of cerebral matter of man and animals (18), earned him the doctorate, as well as the chair (19). He held this post until March 1822 when, following student demonstrations, the faculty was suppressed by le Comte de Villèle, the minister overseeing the medical school. A year later, the faculty was allowed to re-open, but professors considered unfriendly to the régime were excluded, among them Vauquelin, who was known to hold liberal views (20).

Before considering Vauquelin's work on the brain described in his thesis, his analysis of fish roe must be mentioned. A few years after Jordan's illuminating work on brain lipids, Fourcroy and Vauquelin reported the discovery of phosphorus in fish roe. Their preliminary experiments showed that the roe is neutral in reaction; yet the residue from its combustion is strongly acidic. The acid was characterized as phosphoric acid. The authors thought that it must have been formed during combustion. When they resorted to distillation of this fish product, they noticed elemental phosphorus condensing on the walls of the distillation tube.

In other experiments, they extracted fish roe with alcohol and obtained a "soap-like material," which contained phosphorus. The authors proudly state that (21):

The discovery of phosphorus in a combustible state in organized bodies [i.e., living matter] belongs entirely to Messieurs Fourcroy and Vauquelin.

Jordan had identified the new "fatty" substance in brain but had missed the fact that it contained the phosphoric acid he had identified.

Section IV of Vauquelin's thesis is entitled "Examination of the Fatty Matter of the Brain which is Precipitated during the Cooling of the Alcohol used to Extract this Organ." He states that the substance that he isolated was "white, solid but soft, and sticky; that it had a satiny and bright aspect, that it stained paper in the way that oil does" (18). He goes on to describe his first experiment (18):

A portion of this material, which had been dissolved several times in alcohol in order to separate out from it the last of the animal substance [i.e., protein], was burned in a platinum crucible. ... The carbonized residue, washed with distilled water, rendered this fluid very acidic, with its ability to precipitate lime water. The unusual result of this procedure which, evidently indicated the presence of phosphoric acid, made me suspect that this fatty substance contained phosphoric acid in combination.

He continues:

[I]n order to be sure about this ... I diluted some [of the material] with distilled water. [The resulting emulsion] demonstrated no acidity, and did not affect litmus at all.

After describing another experiment, Vauquelin writes:

I believe that I can conclude from these experiments that the brain substance involved here contains neither free phosphoric acid nor ammonium phosphate, and that consequently the acid which forms in the course of combustion has another origin......What is to be concluded from these experiments if not that there is phosphorus combined with fatty material in

the brain and that the former is dissolved along with that fatty substance in alcohol? ... One must necessarily accept that phosphorus is present in the substance of the brain, just as in the roe of fish, as discovered by Fourcroy and myself.

Finally, Vauquelin offers some words of caution:

Although the substance we have described offers a closer relationship to the fats than to all other classes of substances, nevertheless it should not be identified with ordinary fat. It differs from fat mainly by its insolubility in alcohol, by its ability to form crystals, its viscosity, its lesser fusibility, and the black color, which it assumes on melting. Thus, while classifying it among the fatty bodies, it must be regarded as a specific and new substance.

The research that Vauquelin described in his thesis was destined to play a very significant role in the history of neuroscience, as the first complete analysis of the brain by state-of-the-art methods of chemistry. It was not only published in France, but soon appeared in translation in German and English journals (6, 22). Moreover, his extraction of 'white matter' from brain tissue with boiling alcohol, and its precipitation on cooling the solution, became the starting-point for several later investigators of brain chemistry.

Jean-Pierre Couerbe (1805-1867)

One of these investigators was Jean-Pierre Couerbe, a young French chemist hailing from the Bordeaux region. Couerbe trained in chemistry at the School of Pharmacy in Paris, working in several laboratories. For a period he was with Pierre-Joseph Pelletier (1788-1842) but left him in a dispute to work under the toxicologist M. J. B. Orfila (1787-1853) (23).

Couerbe introduced the use of ether as well as alcohol for extraction of lipids of the brain. Moreover, his was the first attempt to analyze the individual constituents making up Vauquelin's 'white matter.' Vauquelin had separated two 'fatty' fractions. Couerbe was able to separate five, one of which was cholesterol. His elemental analysis of the isolated cholesterol conforms very closely to the theoretical, a measure of the purity of his product. Thus, Couerbe demonstrated that it was a normal constituent of the brain. The other fractions were, from the present standpoint, mixtures. However, one of them, which was soluble in ether but not in alcohol or water, was saponifiable and contained phosphorus (24), and so exhibited the properties of phospholipids. This fraction he named 'céphalote' or 'brain wax.' He provided analytical data for this and the other fractions he had isolated. Although his elemental analysis of céphalote does not agree well with that for lecithin, his practice of characterizing each of his isolated fractions distinguished him as "the first to apply organic analysis to brain-products (25)."

Théodore-Nicolas Gobley (1811-1876)

Gobley was born on May 11, 1811, in Paris. He studied pharmacy there as a pupil of Pierre Robiquet (1780-1840),Vauquelin's successor. He received his diploma in pharmacy in 1835 and practiced his profession for many years. In 1842 he was appointed Professeur agrégé at the School of Pharmacy in Paris; the next year he joined the Société de Pharmacie de Paris. In 1861 he was elected to membership in the Academy of Medicine.

Gobley's interests lay not only in laboratory work, but also in carrying out public responsibilities. He took time from his professional career to perform charitable work and to make social contributions as a

member of the Council of Public Health of the Department of the Seine, of the Paris Commission on Unsanitary Housing, and of the Council of the Society for the Promotion of National Industry. He was assiduous in fulfilling these and the other functions he had accepted. He later became administrator, and then vice-president of the welfare offices of his district (26). In addition to these activities he was a member of many scientific societies. Tétry describes Gobley as "devoted, benevolent, and charitable, [a man] without ostentation (27)."

In his scientific work, Gobley dealt with a wide variety of subjects; but the one that concern us now was his research on the composition of hen's egg yolk, brain of several species, and carp organs. In his investigation of the lipid content of the yolk, he isolated lecithin, the first specific phospholipid to be recognized. This was in 1846 (28). He accomplished this by dehydrating the egg yolk and then extracting it with boiling ether or alcohol. Evaporation of the extract yielded an oily liquid and a soft, viscous substance. By hot filtration, the lat-

ter material was retained on the filter paper. The viscous matter was neutral to litmus, but on combustion its ash contained an acid, identified as phosphoric acid. The constituents of the viscous matter that Gobley described at that time were oleic and margaric acids (29), and a specific acid containing phosphorus, namely, phosphoglyceric acid. In addition, there was a base that he at first thought was ammonia.



Theodore-Nicolas Gobley

In 1847 Gobley published a paper in two parts (30) comparing the chemical composition of egg yolk and brain. In it he stated that he had repeated all the egg yolk experiments with brain matter of chicken, sheep, and humans and had found the same fatty acids in the 'viscous matter' extracted from those sources as in egg yolk. However, he was unable to prepare the compound in a pure state. His work was presented to the Academy of Sciences by E. Frémy (1814-1894) who, unfortunately for Gobley, introduced his personal speculations about the composition of the lipids that Gobley had analyzed (31). Three years later, Gobley presented new work dealing with the roe of carp. It is in this paper that he gave the name 'lecithin' (from the Greek 'lekithos,' egg yolk) to what had hitherto been referred to as 'viscous matter' (32). In further

work he identified this new entity also in the milt of carp (33), blood (*i.e.*, in the erythrocytes) (34), in bile (35), and even in the tissues of some lowly invertebrates, such as the sea nettle, the starfish, the sea urchin, medusa, and the sea anemone (36).

As for the basic constituent of lecithin, Gobley drew upon the finding by Adolf Strecker (1822-1871) of choline in bile in 1861-62—that is, a few years after he himself had discovered lecithin in that biological fluid (1856). Strecker, moreover, correctly deduced the structure of lecithin (37). Gobley then concluded that the choline in bile arose through the double decomposition of lecithin (38).

Gobley made numerous contributions to the chemical literature throughout his life, many based upon laboratory research, others of a literary nature such as his articles prepared for various encyclopedias. But it is his studies of animal lipids, particularly his elucidation of the chemistry of the phospholipid lecitihin, for which

he is best remembered. He died at Bagnères-de-Luchon, a spa in the Haute-Garonne, on the first of September 1876, as the result of pulmonary disease.

The 130 years between Hensing's discovery of phosphorus in the brain and Gobley's description of lecithin saw many changes in chemical procedures for the isolation of natural products. The early customary methods of destructive distillation and incineration gave way to solvent extraction and other milder procedures, exemplified in this area of work by Fourcroy's use of aqueous solutions and alcohol. In Vauquelin's hands these techniques led to the recognition of organically bound phosphorus in the brain. Because alcohol was not an ideal solvent for this material, Couerbe's introduction of ether as an extractant advanced the recognition of phosphorus-bound lipid as a novel chemical entity. Gobley concluded the process by characterizing the material, giving it a specific name, and demonstrating its wide distribution in the animal kingdom.

REFERENCES AND NOTES

- 1. Phospholipids include phosphatidylcholine (lecithin), phosphatidylethanolamine, phosphatidylserine (the latter two being known as cephalins), phosphatidyl-myoinositol, diphosphatidylglycerol (e.g. cardiolipin), and phosphatidylsphingosine (sphingomyelin). The phosphatidyl group is diacylglycerophosphoric acid. Phospholipids occur in all living matter but were first recognized in animal tissues.
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- 5. Ref. 3, Tower, p 224-315.
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