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Chemistry, Metabolism and Function

by

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PREFACE

Large numbers of specialised biochemical treatises, monographs, reviews and compilations are now available in the English language. These have already gone a long way towards building Erwin Chargaff's "Tower of Babel made of paper" and some apology is perhaps required for an addition to this structure. As far as we know, however, no single book has been devoted entirely to the phospholipids since the excellent volume published by Wittcoff in 1951. Our respect for this publication has increased enormously since commencing the preparation of our own book because Wittcoff covered every conceivable aspect of the phospholipids known to that date, including their use in the food and other industries.

It is some measure of the advance in knowledge of the chemistry and biochemistry of phospholipids that more than 80% of the references in the present volume are made to studies published since 1951 and only a small proportion therefore overlap with those cited by Wittcoff. Some of the reasons for this advance are given in Chapter 1. We have concerned ourselves almost exclusively with the animal phospholipids and the reasons for this are two-fold. First, the whole phospholipid field is now too large to be covered entirely in a book of reasonable size, and second, the animal phospholipids are the particular interest of the authors. Even so, it is to be expected that contributions to certain foreign publications not readily available to us will have been overlooked and we would be grateful to anyone prepared to point out omissions or misrepresentations.

It is hoped that this book will be of use to research workers in many disciplines, both in the basic sciences and in clinical medicine. The book is not primarily intended for undergraduate students.

It is a pleasure to thank the many people who have assisted in the preparation of this book. Dr. P. B. Bradley, Prof. J. N. Cumings, Dr. A. N. Davison, Dr. J. Dobbing, Dr. J. B. Finean, Dr. R. F. Fletcher, Prof. A. C. Frazer, Dr. G. Hübscher and Dr. K. Walton all read sections of the book and their comments were highly valued. The help given by Miss M. P. Russell and the staff of the Barnes Library, Birmingham Medical School, in hunting obscure

references and making good the errors of the authors is also appreciated. The burden of preparing the manuscript has fallen largely on Miss S. G. Spanner and Mrs. R. Williams who have spent many hours deciphering our handwriting. Miss Spanner has also given freely of her time in the preparation of many of the figures in the text. Acknowledgement for the use of figures from other sources has been made in the text where appropriate.

Birmingham,
June 1964

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CHAPTER 1

INTRODUCTION AND HISTORICAL

In living systems the structures of the cells and tissues are based on large molecules—the proteins, polysaccharides and complex lipids—whilst the organisation of the systems appears to be the function of the nucleic acid complexes. In recent years, detailed studies on proteins, polysaccharides and nucleic acids have demonstrated the patterns of repeating units within an infinite variety of molecular structures. Although most lipid molecules are not as complex or as large as these, a large variety of compounds exists, many of them differing only in the composition of the long-chain fatty acid or aldehyde moieties.

The word lipid (Gr. *lipos*, fat) covers what seems to be an ever-expanding group of compounds, the classification of which has been the subject of some controversy. One of the most widely used classifications is that of Bloor[2] (1925–6), adapted by Deuel in 1951 in his definitive treatise[6]. In this classification lipids are subdivided as follows.

1. Simple lipids
(*a*) Neutral fats (glycerol esters of largely long-chain fatty acids).
(*b*) Waxes (solid esters of long-chain monohydric alcohols).

2. Compound lipids (esters of fatty acids with alcohols, which contain also an additional group)
(*a*) Phospholipids (lipids containing a phosphate residue).
(*b*) Cerebrosides and gangliosides (lipids containing a carbohydrate residue).
(*c*) Sulphatides (lipids containing a sulphate residue).

3. Derived lipids
This group contains products derived from *1* and *2* which still possess lipid-like characteristics. They include fatty acids, long-

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4°. 100 parties de la même matière jetée successivement dans 200 parties de nitrate de potasse fondu, se sont enflammées avec une grande facilité; il ne s'est presque point produit de fumée, tout a été détruit, et il n'est pas resté la plus légère trace de matière charbonneuse.

Le résidu de cette opération, traité de la même manière que celui de la précédente, a donné la même quantité de phosphate de chaux.

Que conclure de ces expériences, si ce n'est qu'il y a du phosphore combiné avec la matière grasse du cerveau, lequel s'est dissous en même tems que cette dernière dans l'alcool?

En effet, on ne trouve dans le résidu de la combustion de cette substance, ni phosphate de chaux ni de magnésie, et les phosphates alcalins auraient trouvé assez d'eau dans la matière cérébrale pour rester en dissolution dans l'alcool, et pour ne pas s'en précipiter par le refroidissement; aussi retrouve-t-on du phosphate de potasse et des phosphates acides de chaux et de magnésie dans le résidu de l'alcool évaporé qui a servi à traiter le cerveau.

Il faut donc nécessairement admettre dans

Fig. 1. A page from one of Vauquelin's papers in which he described the presence of phosphorus bound to fat in the brain[34a]. (Reproduced by kind permission of Masson et Cie., Paris.) This paper is apparently the same as the original publication which was not available to the authors.

chain alcohols, sterols, hydrocarbons (carotenoids, Vitamins D, E, and K) and sphingosine.

In this monograph an attempt is made to assess present knowledge on the phospholipids, more specifically those in animal tissues. A classified list can be found in Chapter 2. In recent years information about the structure and metabolism of the phospholipids has increased considerably, largely as a result of (*a*) new methods of fractionation which have resulted in the preparation of pure substances, and (*b*) methods of chemical synthesis which have produced convenient reference compounds. It is not unlikely that, in the forseeable future, all the phospholipid components of tissues will have been characterised and synthesised; whether their functions will be understood is another matter.

In the following historical sketch, as in similar accounts of the historical development of many biochemical topics, the purely chemical investigations predominate. More elaborate accounts are given by Levene and Rolf[18], Maclean and Maclean[22], Thierfelder and Klenk[30], and Wittcoff[35]. Some interesting incidental historical information about early investigations on brain phospholipids is given in a recent review by Tower[33], whilst a dogmatic and polemical, but highly detailed, commentary on early and mid-nineteenth century investigation is given by Thudichum[31] in his original report to the Privy Council in 1874.

Since phospholipid function in nervous tissue is more obscure than extra-cerebral function it is ironical that the first observation that phosphorus was bound to fatty substances was made on material extracted from brain. This discovery was made by L. N. Vauquelin[34] in 1811 (Fig. 1) who used ethanol as an extractant; ether was not a common solvent at that time and chloroform had not been synthesised. Vauquelin (1763–1829) was a pupil of A. F. de Fourcroy (1755–1809), a well-known French chemist; further details of his activities can be found in Tower's review[33].

In 1834, Couerbe[3] isolated similar substances from brain and his contemporary Frémy[9] (1814–94) named one of them "oleophosphoric acid" (1841). Vauquelin, Couerbe and Frémy all obtained brain material which was soluble in hot alcohol and which was deposited on cooling ("matière blanche", "cérébrote", "acide

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Ueber die chemische Beschaffenheit der Gehirnsubstanz;

von *Oscar Liebreich*,
Assistenten am Schlofslaboratorium zu Tübingen.

So ungemein grofs die Schwierigkeiten sind, die sich bei der chemischen Untersuchung der Nervensubstanz darbieten — wie es alle Forscher einstimmig zugestehen —, schien es doch eines neuen Versuches werth, eine Trennung wenigstens der einigermafsen näher beschriebenen Bestandtheile vorzunehmen, um Genaues über ihr quantitatives Vorkommen zu erfahren.

Wenn ich auch diese Absicht nicht erreicht habe, weil — wie ich später zeigen werde — sie zu erreichen unmöglich ist, so bin ich doch insofern zu einem Resultate gelangt, als ich nachzuweisen vermochte, dafs alle diejenigen Körper, die man als Cerebrin, Cerebrinsäure, Lecithin u. s. w. und als phosphorhaltige Fette bezeichnete, primär nicht im Gehirn existiren. Vielmehr läfst sich ein Theil dieser Körper als reine Zersetzungsproducte eines im todten Gehirn primär vorkommenden Stoffes nachweisen, und ein anderer Theil als nicht hinreichend chemisch characterisirt aus der Reihe wirklich chemischer Individuen zurückweisen.

Es kann hier weder meine Absicht sein, eine historische Darstellung der bisherigen Untersuchungen zu geben, noch die Fehler der Methoden aufzuzählen und zu kritisiren, welche mir bei dem Bestreben, die von früheren Autoren als dem Hirn eigenthümlich beschriebenen Stoffe zu isoliren, aufgestofsen sind. Ich werde mich im Folgenden darauf beschränken, den einen von mir gefundenen Körper und seine nächsten Zersetzungsproducte zu beschreiben und zu erwähnen, in

Fig. 2. The first page of Liebreich's paper[20] in which he put forward the "protagon" concept. (Reproduced with kind permission of Verlag Chemie, Weinheim/Bergstr.)

cérébrique"); this was the source of Liebreich's "protagon" (see p. 6). Gobley (1811–76) isolated from egg yolk and brain a phosphorus-containing lipid[10,11] in 1846–47 which he later (1850) called "lecithin"[12] (Gr. *lekithos*, egg-yolk). In his publication of 1850, Gobley also showed that glycerophosphoric acid could be obtained from lecithin whilst Diaconow[5] who worked in Hoppe-Seyler's laboratory, and Strecker[28] demonstrated the presence of choline in 1868. Strecker[28a] had originally isolated this base from hog bile in 1862 (Gr. *cholĕ*, bile). From these observations Diaconow and Strecker were able to deduce the structure of lecithin, although Strecker alone suggested that choline was attached to the phosphate by ester linkage.

It is however to J. L. W. Thudichum (1828–1901) that phospholipid chemistry owes some of its most considerable advances and, incidentally, some classical terminology. While studying at the University of Giessen (from which he graduated in 1851) he came under the influence of the chemist Liebig. On emigrating to London in 1853 he practised medicine privately before taking teaching appointments in London hospitals, but from 1871 he worked largely in his private laboratory. Here he carried out the fundamental work on the chemical constitution of the brain[31,32] which represents, however, only a portion of his researches. His original publications are difficult to obtain* but a biography of this remarkable man has been published recently[7]; this contains a complete bibliography. A shorter but objective account of Thudichum's contribution to biochemistry has been written by McIlwain[21a].

In the course of his investigations on the brain, he isolated many phospholipid fractions which he attempted to classify by careful analysis and, among other methods, their nitrogen–phosphorus ratio. Many of his substances including, for example, "cephalin" which he separated from brain lipids (Gr. *kephalĕ*, head) and distinguished from lecithin by its relative insolubility in warm ethanol, are now known to be mixtures. In point of fact, although he isolated ethanolamine as a hydrolysis product he did not consider it as a normal constituent of the cephalin but as a decomposition

* Thudichum's book[32] on the chemical constitution of the brain published in 1884 has been expensively reproduced in facsimile with an introduction and glossary by D. L. Drabkin (Archon Books, Hamden, Conn., 1962).

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product of choline. It is typical of the difficulties associated with phospholipid separation that it was not until 1913 that Renall[25] and Baumann[1] simultaneously showed that one of the bases in the cephalin fraction was ethanolamine.

Thudichum also isolated from brain tissue a phospholipid which he designated sphingomyelin "in commemoration of the many enigmas which it presented to the enquirer" (Gr. *sphingein*, to bind tight; *myelos*, marrow). He recognised it as a diaminophospholipid and after alkaline hydrolysis successfully obtained its two constituent bases, sphingosine and choline, in addition to phosphoric acid and a fatty acid. It is noteworthy that he realised that "protagon" (Gr. *protos*, first; *agonistes*, a combatant), thought by Liebreich[20] (Fig. 2) to be the mother substance of all phospholipids, was not a distinctive substance, in disagreement with the theories of many, but not all, German chemists. For over twenty years (1874–96) Thudichum produced his evidence and bitterly stated his case but his assertions were not vindicated until Rosenheim and Tebb showed that protagon was a mixture of sphingomyelin and cerebroside[26]. The problem of protagon, although now of academic interest, had considerable significance more than fifty years ago and its ramifications were well documented by Maclean and Maclean[22] in 1927.

It is perhaps not unfair to say that from about 1905 until the late nineteen thirties, knowledge of the chemical nature of phospholipids did not greatly advance. Two or three investigators in this period should, however, be mentioned. Levene carried out extensive investigations on the separation and purification of sphingomyelin and other phospholipids and identified some of the fatty acids present[18]. He was the first to obtain a relatively pure sphingomyelin and showed that its hydrolysis products were exclusively choline, sphingosine, phosphoric acid and fatty acids, one of which was lignoceric acid[16,17]. Klenk showed in 1929 that sphingosine had a C_{18} structure[14]; he has subsequently made important contributions to sphingolipid and phospholipid chemistry as will be indicated in subsequent Chapters. In spite of the extent of these investigations, however, the structure of sphingomyelin was not established until 1953 (see Chapter 2).

Because of the lack of adequate structural information and

reference compounds, investigations of metabolism were sporadic. Advances made in the same period in, for example, the structure of carbohydrates and their metabolic intermediates is in striking contrast with progress in phospholipid biochemistry; yet many of the intermediates in carbohydrate metabolism are distinctly elusive entities, often present in small amounts. Lipids, even minor ones, are relatively gross components of the tissues.

The difficulties lay largely in the absence of adequate methods of fractionation and the hazards involved in obtaining components which would crystallise. In 1916 Levene and West[19] stated ". . . . the experience on sphingomyelin has shown that often thirty recrystallisations are required before the substance is obtained in a sufficient degree of purity". The phospholipids do not greatly differ from one another in many physical properties and the fatty acid or aldehyde components confer the properties of intractable oils on many of the impure specimens.

Most of the early methods of fractionation of phospholipid mixtures depended upon the differential solubility of their components. It was observed, for example, that sphingomyelin is virtually insoluble in ether and that lecithin is more soluble in warm ethanol than the components of "cephalin". Folch[8] brilliantly exploited relative solubilities in 1942 when he successfully separated brain cephalin into three fractions containing respectively ethanolamine, serine and inositol. It is a further comment on the earlier difficulties that in 1914 MacArthur[21] had demonstrated the presence of α-aminocarboxylic acid nitrogen in cephalin but another thirty years were to elapse before the source of this nitrogen was identified.

Greater success was obtained in the nineteen thirties in ascertaining the distribution of lipid components in a sample or in tissues by the estimation of hydrolysis products which were water-soluble and/or capable of chemical determination. Thus the determination of choline became important and its estimation as the reineckate is still of considerable value (see Chapter 3). Phosphorus and nitrogen determinations had been used earlier for characterisation. It was more difficult to determine glycerol, ethanolamine and serine and in fact, methods for their determination are still being revised or devised. Schmidt and his co-workers[27] devised a method in 1946 whereby the lability of certain phospholipids to mild alkali treatment

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