

Chapter 1

Historical Landmarks in the Discovery of Chitin



Grégorio Crini

Abstract In 1799, Hatchett decalcified shells of crabs, lobsters, prawns, and crayfish with mineral acids, observing that “they produced a moderate effervescence and in a short time were found to be soft and plastic of a yellowish color and like a cartilage, which retained the original figure”. Although this is the first mention of calcified chitin in invertebrates, the discovery of chitin is usually attributed to Braconnot in 1811 who discovered it from fungi, and its recognition to Odier in 1823 who obtained a hornlike material after treatment of cockchafer elytra with potassium hydroxide. Chitin first named *fongine* by Braconnot and then *chitine* by Odier. Children revealed the nitrogenous nature of chitin in 1824. The history of chitosan, the main derivative of chitin, dates back to 1859 with the work of Rouget. The name of chitosan was, however, introduced in 1894 by Hoppe-Seyler. In 1876, Ledderhose hydrolyzed arthropod chitin and discovered *glykosamin*, the first derivative of chitin. The main aim of this chapter is to describe the 220 years of the development of chitin. I have roughly divided it into five periods: discovery from 1799 to 1894, a period of confusion and controversy from 1894 to 1930, exploration in 1930–1950, a period of doubt from 1950 to 1970, and finally the period of application from 1970 to the present day. The different periods are illustrated by considering examples of studies that appeared in the literature and in particular those of several great scientists who have left their mark on the history of this polysaccharide. Although this historic review cannot hope to be exhaustive, it does highlight the work of those researchers who have contributed to the development of our knowledge of chitin throughout the 220 years of its history.

Keywords Chitin · Chitosan · History · Discovery · Braconnot · Controversy · Exploration · Period of doubt · Period of application

G. Crini (✉)

Chrono-Environnement, UMR 6249, Université Bourgogne Franche-Comté,
Besançon, France

e-mail: gregorio.crimi@univ-fcomte.fr

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1.1 Introduction

Chitin is the most abundant of the renewable polysaccharides in the marine environment and one of the most abundant on Earth after cellulose. Cellulose is the significant structural polymer in the primary cell walls of the plant cells while chitin is the main structural polymer found in the fungal cell wall. Chitin is also present on exoskeletons of arthropods and insects. Cellulose and chitin are both β -1,4 linked polysaccharides composed respectively of glucose and N-acetylglucosamine, i.e. 2-acetamido-2-deoxy-D-glucose. These two polysaccharides are used industrially in many different applications. Because of their similarity, research on chitin and cellulose has often been interwoven, with reciprocal advantages, e.g. studies on constitution, chemistry, biosynthesis, and mostly (micro-fibrillar) structure.

The history of chitin began in France, in the beginning nineteenth century, with the work of the chemist Henri Braconnot. Braconnot in 1811 named the insoluble residue remaining after the extraction of fungi with water, alcohol, and dilute alkali *fongine*/fungine (Braconnot 1811a, b). He discovered chitin polysaccharide from fungi, preceding cellulose by about 30 years. However, chitin was probably discovered by Charles Hatchett, an English chemist, who, during his experiments on the shells of marine animals, reported in 1799 the presence in the cuticle of “a material particularly resistant to usual chemical” (Hatchett 1799). However, there is no indication that he was aware of what he had done.

The history of chitosan, the main derivative of chitin, dates back to 1859 with the work of Charles Rouget who reported that treatment of *chitine* with concentrated caustic potash solution under reflux gave a new “*chitine modifiée*”, modified chitin, and this treatment rendered it soluble in organic acids (Rouget 1859). The name of chitosan was, however, introduced in 1894 by Felix Hoppe-Seyler for an acid-soluble derivative of chitin prepared from the treatment of the shell of crabs, scorpions and spiders (Hoppe-Seyler 1894).

This chapter reviews the main historical landmarks in the discovery and the development of chitin, and also chitosan, its main derivative, reported during the period 1799–2018. I have roughly divided it into five periods: discovery from 1799 to 1894, a period of confusion and controversy from 1894 to 1930, exploration in 1930–1950, a period of doubt from 1950 to 1970, and finally the period of application from 1970 to the present day. The different periods are illustrated by considering examples of studies that appeared in the literature and in particular those of several great scientists who have left their mark on the history of these polysaccharides.

1.2 Discovery: 1799–1894

The first period, from 1799 to 1894, covers the discovery of chitin and chitosan. The names that marked this period most were Charles Hatchett, Henri Braconnot, Auguste Odier, John George Children, Charles Rouget, Georg Ledderhose, and Felix Hoppe-Seyler.

1.2.1 *Charles Hatchett*

Charles Hatchett (1765–1847) was an English chemist and a self-formed mineralogist and analytical chemist (Johnson 1803; Good et al. 1813; Smedley et al. 1845; Wisniak 2015). In 1799, Hatchett decalcified shells of crabs, lobsters, prawns, and crayfish with mineral acids. In a 20-page memory, Hatchett described a material particularly resistant to usual chemical (Hatchett 1799). In June 13, 1799, he wrote that “it appears that immersion of the shell in acetous, or in dilute nitric acid, afforded carbonate and phosphate of lime, the former, however, in largest quantity”. This is the first mention of calcified chitin in invertebrates (Smedley et al. 1845). In February 1800, in the *Journal of Natural Philosophy, Chemistry, and the Arts* (Fig. 1.1), Hatchett wrote that “Pieces of this substance, taken from various parts of those animals, was at different times immersed in acetous and in diluted nitric acid; those which had been placed in the diluted nitric acid produced a moderate effervescence, and in a short time were found to be soft and elastic, of a yellowish white colour, and like a cartilage, which retained the original figure” (Hatchett 1800a, b). However, Hatchett did not push his scientific investigations any further, preferring to become a noted collector of books, paintings, musical instruments, and musical manuscripts (Wisniak 2015).

1.2.2 *Henri Braconnot*

Henri Braconnot (1780–1855) was a French chemist, Director of the *Jardin Botanique* of Nancy (Fig. 1.2). His work was essentially devoted to the extractive principles of natural vegetables and various aspects of the chemistry and physiology of natural substances including carbohydrates and alkaloids. Braconnot’s scientific career covers the period 1806 to 1854, in which he published over 110 *mémoires* (Simonin 1856, 1870; Nicklès 1856a, b; Donzelot 1953; Prévost and D’Amat 1956; Labrude 1997; Labrude and Becq 2003; Wisniak 2007; Nwe et al. 2011; Muzzarelli et al. 2012).

In 1811, Braconnot discovered an alkaline-insoluble fraction from fungi such as *Agaricus volvaceus*, by treatment with dilute warm alkali (Braconnot 1811a, b, c). He gave it the name of *fongine* (fungine), a substance “*d’une nature particulière*”

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AND
THE ARTS.

FEBRUARY 1800.

Experiments and Observations on Shell and Bone. By CHARLES HATCHETT, Esq. F. R. S.*

SOME experiments, which I lately made at the request of Mr. Home, and which he has done me the honor to mention in his ingenious paper on the teeth of granivorous quadrupeds, induced me to turn my attention more particularly to the chemical examination of shell and bone, especially as the former appeared to have been hitherto much neglected.

The time since these experiments were begun, has not been sufficient to enable me to enter into all the minutiae of the chemical analysis of these substances; but as some remarkable facts were ascertained, I have now ventured to bring them forward, with the addition of some observations, although as yet the whole is little more than a very imperfect outline.

The first of these experiments were made on the shells of marine animals; and to avoid repetition and prolixity, I shall, in a great measure, once for all, describe the menstrua, the precipitants, and the mode of operation.

When shells were examined they were immersed in acetous acid, or nitric acid diluted, according to circumstances, with 4, 5, 6, or more parts of distilled water; and the solution was always made without heat.

Fig. 1.1 Extract of the article by Hatchett on his experiments and observations on shell and bone in February 1800. (Hatchett 1800a)

(Fig. 1.3). Braconnot analyzed the nitrogen content in the liquid obtained by distillation of this fraction, and found that the liquid contained acetate of ammonia contaminated with oil. This fraction also produced acetic acid by degradation with concentrated sulfuric acid (Braconnot 1811b). In a later paper, Braconnot observed that his *fongine* had many different consistencies, more or less soft, leathery, cartilaginous, or cork-like, indicating that there were differences in the proportion of its components (Braconnot, 1813). He repeatedly stated that his *fongine* differed from woody materials, containing more nitrogen than wood but much less than wheat protein or in animal materials.

Fig. 1.2 Professor Henri Braconnot, 1780–1855. (Source: Simonin 1856)



1.2.3 Auguste Odier

In August 17, 1821, Auguste Odier presented a *mémoire* at the *Société d'Histoire Naturelle de Paris* on a new substance found in the elytra of insects (Fig. 1.4), published in 1823 (Odier 1823; Bounoure 1919). Odier isolated an insoluble alkaline-insoluble fraction from cockchafer (May beetle) by repeated treatments with hot caustic potash solutions. He gave it the name of *chitine*, from Greek word chiton (“χιτόν”), meaning a covering, tunic or envelope (Odier 1823; Straus-Durckheim 1828; Payen 1843). After several treatments, Odier observed that *chitine* was stable in external form and soluble in sulphuric acid “with the assistance of heat” but did not become yellow by the action of nitric acid. However, *chitine* was dissolved when digested in it with heat. At the same time, a similar substance named *entomaderme* was also observed by the young student Lassaigne (Straus-Durckheim 1828; Latreille 1831). Odier concluded (1) “*la chitine est une substance particulière, fort curieuse*” and (2) “*il est fort remarquable de retrouver dans la charpente des insectes la même substance qui forme celle des végétaux*”. Indeed, he thought the frameworks of insects and of plants were composed of the same substance, cellulose.

Odier noted that *chitine* made up only a relatively small part of the insect cuticle: the elytra of *Melolontha* contained about 29%. There was a certain amount of ash

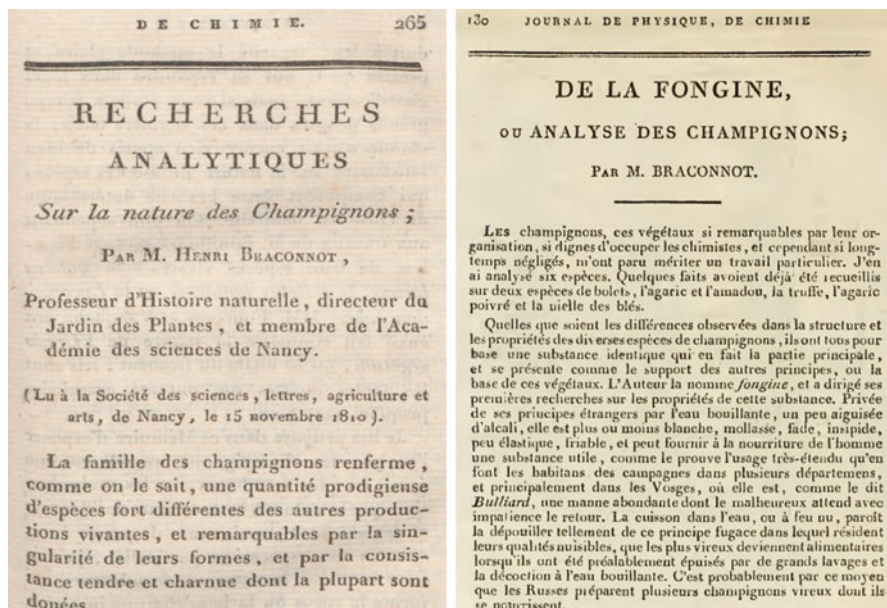


Fig. 1.3 First page of two *mémoires* of Braconnot published in 1911 (left) in the journal *Annales de Chimie* (Braconnot 1811a) where he described his experiments, and (right) in the *Journal de Physique, de Chimie, d'Histoire Naturelle et des Arts* where he proposed the name of *fongine* (Braconnot 1811c)

and some oils. The bulk of the non-chitinous substance was considered by him to be protein (Straus-Durckheim 1828; Latreille 1831). Odier also identified his *chitine* as present in demineralized crab carapace and suggested that it was the basic material of the exoskeletons of all insects, and possibly, the arachnids (Odier 1823; Straus-Durckheim 1828; Latreille 1831; Bounoure 1919). He tested for nitrogen in this residue and came to the conclusion that it did not contain nitrogen (Odier 1823). In 1855, Edmond Frémy (1814–1894), another French chemist, reported a similar conclusion for his *chitinelfongine*, named by him *metacellulose* (Frémy 1855). The nitrogenous nature of *chitine* was revealed by the experiments of Children (1824). However, the discovery that nitrogen was present in chitin of insect origin is usually attributed to both Lassaigne (1843a, b, c) and Payen (1843). Both Straus-Durckheim (1828) and Latreille (1831) previously indicated that the presence of nitrogen was highlighted by the young student Lassaigne in *entomaderme (chitine)*.

MÉMOIRE
SUR
LA COMPOSITION CHIMIQUE
DES PARTIES CORNÉES DES INSECTES.
PAR M. AUGUSTE ODIER.
(LU DANS LA SÉANCE DU 17 AOUT 1821.)

DEPUIS assez long-temps, des expériences souvent répétées nous ont fait connaître la composition chimique des os des vertébrés du premier ordre. Quelques savans, curieux de comparer la charpente osseuse des animaux inférieure à celle des êtres plus élevés dans l'échelle zoologique, ont appliqué leurs connaissances à l'analyse des premiers. C'est ainsi que nous avons appris des chimistes la composition des os des poissons, du test des mollusques, des crustacées et des parties dures des zoophytes.

Mais dans ces divers travaux on n'avait encore jamais pris pour objet de recherches chimiques les parties solides du corps des insectes, et l'on se contentait de les assimiler aux matières animales auxquelles elles ressemblent le plus, soit pour les fonctions, soit pour l'apparence physique : de-là les uns, les comparant aux os

Fig. 1.4 First page of the *mémoire* presented by Odier at the *Société d'Histoire Naturelle de Paris* in August 17, 1821. (Odier 1823)

1.2.4 John George Children

Odier's paper was promptly published in an English translation for the *Zoological Journal* by Children in 1824 (Fig. 1.5). John George Children (1777–1852) was a British chemist, mineralogist and zoologist at the British Museum. He felt that Odier's conclusion that the *chitine* was nitrogen free because the products of its dry distillation had no effect on test papers was open to debate (Smedley et al. 1845). It is important to note that Children was not aware of Braconnot's much earlier work on *fongine*.

ART. XV.—*Memoir on the Chemical Composition of the Corneous parts of Insects.* By M. AUGUSTUS ODIER.*
 With some additional remarks and experiments, by J. G. CHILDREN, Esq. F. R. S. L. S. &c.

Experiments, often repeated, have long since taught us the chemical composition of the bones of vertebrated animals of the first order. The skeletons of animals of inferior rank in the zoological scale, have also been subjected to chemical analysis, for the purpose of comparison with those of the former, whence we have obtained a knowledge of the component parts of the bones of fishes, the shells of mollusca and crustacea, and the hard portions of the zoophyta.

The solid parts of the bodies of insects, however, have never been made the subject of chemical research; naturalists have been satisfied with likening them to those animal substances which they most nearly resemble either in their functions, or natural appearance; hence some, comparing them to the bones of vertebrated animals, have named them osseous parts, and have even ventured to call the whole assemblage the skeleton of the insects; others, on the contrary, likening them to the integuments of vertebrated animals, have considered them as hardened skin, or a matter analogous to horn.

In undertaking to investigate the chemical composition of these organs, my object is not to support either of these two opinions; I shall examine the substances with the sole view of ascertaining their composition, without seeking to associate them with any particular organ of the superior animals.

The analysis of some *Crustacea*, by M. M. Merait, Guillot, and Chevreul, are the only works that have been published, as far as I know, on animals approaching the class of insects; and amongst these much discrepancy exists as to the composition of their integuments.*

* Translated from the "Memoires de la Societ  d'hist. nat. de Paris," vol. 1.

† Has M. Odier never heard of Mr. Hatchett's elaborate "Experiments and Observations on Shell and Bone," or his "Chemical Experiments on Zoophyta?" See Phil. Trans. 1799 and 1800. C.

Fig. 1.5 First page of the article of Children where he translated the Odier's paper and described his own experiments in 1824. (Children 1824)

Children, repeating the same experiments, also extracted an alkaline-insoluble fraction from May bug elytra. He observed that “during the action of the alkali, a slight disengagement of ammonia was perceptible”. Children analyzed by elemental analysis the residue left after repeated extractions with strong potassium hydroxide solution and found substantial quantities of nitrogen (11.05% and 9.54% in two analyses), giving the empirical formula $C_{11}H_{17}O_7N_2$. Children also found in the cantharides “a small portion of silica and magnesia, and a slight trace of manganese”.

Children published his own observations in an appendix in the same journal (Children 1824). In this paper, he suggested that “Odier’s test could have failed if the volatile acetic acid was evolved simultaneously with the ammonia”. The resulting neutralization would give negative tests for ammonia and this had led Odier to conclude that nitrogen was absent. This suggestion appears to have been made in ignorance of Braconnot’s work in which the evolution of acetic acid was reported. However, since the samples burnt by Odier had only been extracted with boiling water and not with hot caustic potash solutions, they would have contained a considerable amount of protein which should have given a positive test for nitrogen on burning. Hence, Children’s explanation cannot be correct and the reason for Odier’s negative result is unresolved (Roberts 1992). From the description of the process, it is probable that Children isolated chitosan rather than chitin (Roberts 1992).

1.2.5 Charles Rouget

The history of chitosan dates back to 1859 with the work of the French physiologist Rouget. Charles Marie Benjamin Rouget (1824–1904) was Professor of Physiology at the *Muséum d’Histoire Naturelle* in Paris (Gréhant 1904a, b). In 1859, Rouget found that boiling *chitine* in a concentrated potassium hydroxide solution under reflux rendered it soluble in dilute solutions of organic acids (Rouget 1859). This new product also gave a different color on treatment with an acidified iodine solution than did the original *chitine* (Fig. 1.6). Indeed, mixtures of iodine and zinc chloride gave blue or violaceous colors with *chitine*. Rouget was the first to describe the deacetylation of chitin, opening new possibilities for its use. He gave the name of the product “*chitine modifiée*”, modified chitin. However, it was not until 1894 that Hoppe-Seyler named the tailored chitin, chitosan.

1.2.6 Georg Ledderhose

Georg Ledderhose (1855–1925) studied medicine in Strassburg as a pupil of Georg Albert Lücke (1829–1894). In 1875, Ledderhose, studying the hydrolysis of *chitine* with concentrated HCl, discovered *glykosamin* “*unter der gütigen leitung des herrn Hoppe-Seyler*”, i.e. with the kind collaboration of Hoppe-Seyler, in Strassburg (Ledderhose 1876, 1878, 1880a, b). This was the topic of his doctoral thesis

MÉMOIRES PRÉSENTÉS.

CHIMIE HISTOLOGIQUE. — *Des substances amylacées dans les tissus des animaux, spécialement des Articulés (chitine); par M. Ch. ROUGET.* (Extrait par l'auteur.)

(Commissaires, MM. Pelouze, Milne Edwards, Rayer.)

« Ayant constaté et établi (1) que la substance amylacée signalée dans l'arnios ou le placenta des Mammifères n'est pas le produit d'organes particuliers, qu'elle n'est pas renfermée dans des *cellules glycogènes* spéciales, mais dans les cellules épithéliales mêmes de ces membranes, plus ou moins modifiées, j'ai été conduit à chercher cette substance dans d'autres épithéliums; et je l'ai trouvée, en effet, dans les cellules épidermiques de la peau, du voile du palais, de la langue, dans l'épithélium de l'estomac, dans toutes les cellules cylindriques du revêtement épithélial des villosités de l'intestin grêle et de la surface du gros intestin. J'ai aussi montré le premier que chez certaines espèces, chez les Cobayes en particulier, trois ou quatre jours au plus avant la naissance, tout l'épithélium de l'intestin est rempli de substance amylacée, bien qu'en même temps le foie, depuis longtemps déjà

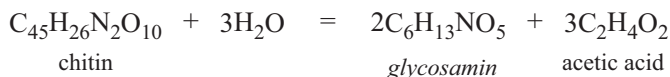
Fig. 1.6 First page of the memory of Rouget, presented at the *Académie des Sciences* Paris, where he described his *chitine modifiée* in 1859. (Rouget 1859)

presented in 1880 at the *Kaiser Wilhelms Universität*, Strassburg. Ledderhose subsequently worked as a surgeon at the Strassburg hospital and became Professor Extraordinary of Surgery in Strassburg in 1891. Later, he worked in Munich, where he became Honorary Professor.

In 1875, Ledderhose was working during the summer semester in the laboratory of his uncle, Friedrich Wöhler (1800–1882), at Göttingen (Foster and Webber 1961; Brimacombe and Webber 1964). One day, Wöhler had lobsters for lunch and, bringing back the shell to the laboratory, he gave it to his nephew. He told him to “find out what this was”. The young Ledderhose treated lobsters with hot concentrated hydrochloric with the aim to identify the structure of the products. He found that the claws and the shells dissolved in this solution and that on cooling the solution yielded characteristic crystals. In collaboration with Hoppe-Seyler, Ledderhose identified the crystalline compound as a new nitrogen-containing sugar which he named *glykosamin/glycosamin*. In 1876, he published his first results in the journal *Berichte der Deutschen Chemischen Gesellschaft* (Ledderhose 1876) where he described the presence of nitrogen (6.49%) and proposed the formula described in Scheme 1.1. The new crystalline product differed from glucose in having an amine group in place of one hydroxyl. Later, Ledderhose also found that acetic acid was a product of hydrolysis of arthropod chitin (Ledderhose 1878, 1880). He estimated



Scheme 1.1 *Glykosamin*: Structural formula proposed by Ledderhose (1876)



Scheme 1.2 Decomposition of chitin according to Ledderhose (1878)

the quantity of products and arrived at the chemical reaction described in Scheme 1.2, confirming the structural formula $\text{C}_6\text{H}_{13}\text{NO}_5$. Ledderhose noticed, together with the acetic acid, small quantities of other volatile fatty acids, especially formic and butyric. Similar results were previously published by Schmidt (1845) and Städeler (1859). However, Ledderhose did not prove that glucosamine and acetic acid were produced in equimolar amounts. Indeed, the stoichiometry of reaction was only determined in 1912 by Brach and von Fürth (1912).

Glykosamin/glycosamin was the first amino sugar isolated in 1876 by Ledderhose. The term glucosamine was nevertheless coined by Tiemann in 1884 (Tiemann 1884). Moreover, the presence of *glykosamin*/glucosamine as the repeated unit of chitin was finally confirmed a few years later by the works of Tiemann (Tiemann 1884; Tiemann and Landolt 1886), Schmiedeberg (1891), Winterstein (1893, 1894a, b, 1894c, 1895a, b, c, d), and Gilson (1894a, b, c, 1895a, b).

1.2.7 Felix Hoppe-Seyler

Felix Hoppe-Seyler (1825–1895) was a German physiologist and chemist. Ernst Felix Immanuel Hoppe, his name at birth, was also a pioneer of biochemistry and molecular biology (Baumann and Kossel 1895a; Noyer-Weidner and Schaffner 1995). In 1846, Hoppe enrolled as a medical student at Halle, where he began chemical laboratory work. A year later, he worked in the laboratory of Ernst Heinrich Weber (1795–1878) in Leipzig and studied chemistry, medicine and physiology with Otto Linné Erdmann (1804–1869) and with Karl Gotthelf Lehmann (1812–1863). Hoppe then completed his medical studies in Berlin, where he presented his doctoral dissertation in 1850 on the chemical and histological aspects of cartilage structure (Baumann and Kossel 1895a, b; Fruton 1990). In 1861, Hoppe-Seyler became full Professor of Applied Chemistry in the medical faculty of Tübingen and in 1872 full Professor in Physiological Chemistry and Hygiene in the medical faculty of the newly established German university in Strassburg.

In 1894, Hoppe-Seyler treated the shells of crabs, scorpions and spiders with potassium hydroxide at 180 °C and found a “new” product (Hoppe-Seyler 1894, 1895). Hoppe-Seyler, who did not refer to the *chitine modifiée* of Rouget, gave it the name of chitosan (Fig. 1.7) and pointed out different observations (Hoppe-Seyler 1894): i) this product was readily soluble in dilute acetic acid, in agreement with the observation by Rouget (1859), and in hydrochloric acid solution, ii) it could be precipitated from such solutions by addition of alkali; and iii) it began decomposing

610. F. Hoppe-Seyler. Ueber Chitin und Cellulose.

(Eingegangen am 4. December; mitgeth. in der Sitzung von Hrn. A. Pinner.)

In diesen Berichten 27, Heft 17, S. 3113—3115 ist von E. Winterstein soeben eine Mittheilung, betitelt »Ueber ein stickstoffhaltiges Spaltungsproduct der Pilzcellulose« veröffentlicht, die mir besonders durch ihren letzten Satz Veranlassung zu folgenden Bemerkungen giebt:

Unter Benutzung einer vor mehreren Jahren beschriebenen Isolirungsmethode, welche von Winterstein auch in der citirten Mittheilung erwähnt ist, habe ich mich seit längerer Zeit mit den der Cellulose verwandten Kohlehydraten von Thieren und Pflanzen beschäftigt und einige Resultate erhalten, deren kurze vorläufige Schilderung wohl jetzt zweckmässig sein wird, während die ausführlichen Mittheilungen erst später gegeben werden können.

Das Tunicin der Tunicaten hat sich auch bei der Behandlung mit Aetzkali bis 180° als übereinstimmend mit gewöhnlicher Cellulose erwiesen, dagegen hat das Chitin der Gliederthiere (untersucht wurden Panzer von Insecten, Krebsen, Scorpionen, Spinnen) eine recht merkwürdige Abweichung gezeigt. Während bei dem Erhitzen mit Aetzkali und ein wenig Wasser im Oelbade bis 180° (als Maximum) die Formen der Chitingewebe so wenig wie die Zellengewebe der Pflanzen bis hinab zu den Algen eine wesentliche Aenderung erkennen lassen, wird das Chitingewebe nach dieser Behandlung und sorgfältigem Auswaschen des Aetzkalis mit kaltem Wasser leicht löslich in verdünnter Essigsäure zur klaren Flüssigkeit gefunden und durch Alkalilauge wird aus dieser Lösung ein reichlicher voluminöser Niederschlag gefällt. Der Stickstoffgehalt des Chitins ist bei dieser Behandlung unverändert geblieben, aber im aufgelösten Aetzkali, mit dem das Chitin erhitzt war, fand sich Essigsäure und zwar so rein, dass nach Uebersättigen mit Schwefelsäure aus dem Destillate sofort das Baryumsalz und aus diesem das Silbersalz von berechneter Zusammensetzung erhalten wurden. Das Umwandlungsproduct des Chitins, welches neben Essigsäure entsteht, sich in Essigsäure, auch in äusserst verdünnter Salzsäure sehr leicht löst, dem ich den vorläufigen Namen Chitosan gegeben habe, zeigt insofern basische Eigenschaften, als es sich mit den Säuren leicht verbindet, beim Verdunsten der wässrigen Lösung der salzsauren Verbindung diese in quadratischen Krystallen liefert, die

Fig. 1.7 First page of the article of Hoppe-Seyler published in 1894 in the journal *Berichte* where he introduced the name chitosan. (Hoppe-Seyler 1894)

at temperature 184 °C and was stated, surprisingly, to have the same nitrogen content as the original chitin. One year later his pupil Araki (1895) and later Löwy (1909) also reported similar conclusions.

Using Schmiedeberg's view of the constitution of chitin (Schmiedeberg 1891), Hoppe-Seyler clearly demonstrated the relationship between chitin and chitosan. When chitosan is treated with concentrated hydrochloric acid it, like chitin, yielded glucosamine (Hoppe-Seyler 1894, 1895). If heated with acetic anhydride, it yielded

a substance resembling chitin, which, when heated with potash at 180 °C, was resolved into chitosan and acetic acid.

At the same time, the product described by Hoppe-Seyler as a partially deacetylated, acid-soluble derivative of chitin was also prepared from fungal material by both Winterstein (1893, 1894a, b, 1895a, b, c, d) and Gilson (1894a, b, c). Hoppe-Seyler however claimed priority (Hoppe-Seyler 1894).

1.3 A Period of Confusion and Controversy: 1894–1930

From 1894 to 1930, chitin entered in a period of confusion and controversy. The names that marked this period most were Ernst Winterstein, Eugène Gilson, Sigmund Fränkel, Emil Fischer, James C. Irvine, Paul Karrer, and Albert Hofmann. During this period, research on chitin was mostly directed toward the study of its occurrence in living organisms, its determination and its chemistry. These works were even marred by frequently contradictory results and hot debate between the numerous and different laboratories, due mainly to confusion arising from the terminology of the different polysaccharides studied during the 19th Century, the lack of a systematic nomenclature, and also the lack of certainty concerning their structure (Haworth 1946; Bell 1949; Tracey 1957).

1.3.1 Nomenclature

Chitin first named *fongine* by Braconnot (1811) and then *chitine* by Odier (1823). However, other names have been proposed but have not gained acceptance and/or led extensive debate. These include *elytrine* by Children (1824), *entomaderme* by Lassaigne (1843a, b), *metacellulose* by Frémy (1855), fungus-cellulose, fungo-cellulose, fungal cellulose, or *pilzcellulose* by de Bary (1887) and Winterstein (1893), *entomeiline* by Packard (1886, 1898), *pupine* by Griffiths (1892a, b), and later *mycetin* by Ilkewitsch (1908). The American entomologists frequently used the term *chitinous* or *chitinized* in morphological or taxonomic descriptions (Ferris and Chamberlin 1928; Campbell 1929).

1.3.2 Ernst Winterstein

In August 1893, the Swiss chemist Ernst Winterstein (1865–1949), who removed fats and proteins from fungus, e.g. *Boletus edulis*, *Agaricus campestris*, and *Morchella esculenta*, found that the residue was insoluble in Schweitzer's reagent (Winterstein 1893). He concluded that it was a cellulose differing from that in tissues of higher plants and named it "fungus cellulose"/*pilzcellulose*. Winterstein also

reported that a nitrogenous substance and acetic acid were among the products of the acid hydrolysis of fungal chitin (Winterstein 1893). The 9 November of the next year, Gilson (1894a), in France, reported the presence of chitin in fungi, studied its chemistry and its conversion to *mycosine*/mycosin (chitosan). He also noted the presence of glucosamine, a “new nitrogenous substance”. Six days later, Winterstein published another paper dealing with “fungus cellulose”, the nitrogen-containing material obtained from the same fungi by fusion with caustic potash solution at 180 °C (Winterstein 1894a). In this work, Winterstein also confirmed identification of glucosamine in the products when heated with hydrochloric acid, the same glucosamine previously described by Ledderhose. The next year, Winterstein showed that mycosin from “fungus cellulose” was decomposed in acid solution into D-glucose, other hexoses and then into acetic acid and an undetermined nitrogenous organic substance (Winterstein 1895c, 1895d). When heated with concentrated hydrochloric acid, it yielded a crystallisable fission product, which proved identical with the hydrochloride of chitosamine, $C_6H_{11}O_3NH_2$, HCl, at that time erroneously termed glucosamine. The same behavior was exhibited by *chitine*, the substance discovered by Odier. As also shown by Ledderhose in 1876, this substance furnished under similar treatment the glucosamine, and that too in the state of hydrochloride. Discussion of whether fungal cellulose was identical with the cellulose of higher plants had been vigorous, the evidence adduced being based on solubility behavior and staining reactions summarized by Winterstein (1894b). The use of name cellulose with reference to fungal chitin as proposed by Winterstein continued for several years.

1.3.3 Eugène Gilson

The chemical similarity of fungal and animals chitins is attributed to Gilson. Eugène Gilson (1862–1908), a pupil of Hoppe-Seyler, was Professor at the University of Gand (Leboucq 1913). Gilson believed that fungus tissue did not contain cellulose. In 1893, Gilson was unable to obtain crystalline cellulose from *Mucor vulgaris*, *Thamnidium vulgare*, and *Agaricus campestris*, while he succeeded easily with plant tissue (Gilson 1893). One year later, Gilson studied the presence of chitin in fungi (Gilson 1894a, b, c) and noted that its elemental composition was in close agreement with previously reported analyses for chitin of insect origin. Gilson also noted that the residue obtained after treating certain fungi with dilute sulphuric acid, and then dilute sodium hydroxide under reflux gave glucosamine on hydrolysis with hydrochloric acid and that, just as in the case of chitin, acetic acid was produced during hydrolysis.

By fusing cell preparations, e.g. *Agaricus campestris*, ergot of rye *Secale cornutum*, with caustic potash at 180 °C, Gilson (1894a, b, c) also obtained a residue (in sulphate or chlorhydrate form), not of cellulose but a substance insoluble in Schweitzer’ reagent, and to which he gave the name of *mycosine*/mycosin with the formula $C^{14}H^{28}Az^2O^{10}$. One year later, Araki (1895), studying the formation of

chitosan/mycosin from chitin, proposed the composition $C^{18}H^{30}N^2O^{10}$ for chitosan. Gilson showed that *mycosine* was soluble in 2 to 3 per cent hydrochloric acid or in very dilute acetic acid (Gilson 1895). A solution of iodine in potassium iodide, containing a trace of free acid, gave a reddish violet stain. Zinc-iodo-chloride solution varied in action in accordance with the amount of zinc chloride present, 50% producing a blue to blue-violet coloration. These reactions closely resembled those of cellulose. Gilson was also among the early researchers to point out that chitin may be associated with other carbohydrate materials and substances analogous or identical to those found in phanerogams. The name *mycosine*/mycosin, like the alternative names for chitin, has never come into general usage.

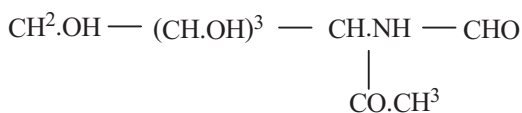
1.3.4 Sigmund Fränkel

Sigmund Fränkel (1868–1939) was a Polish-born physiological chemist and pharmacist who worked in Vienna. Fränkel studied medicine at the Universities of Prague, Freiburg, and Vienna. He played an important role in research into chitin chemistry (Fränkel 1898; Fränkel and Jellinek 1927; Fränkel and Kelly 1901a, b, 1903). In 1901, using a milder acid hydrolysis of purified chitin (cold concentrated H_2SO_4 for two days, room temperature), Fränkel and his colleague, Agnes Kelly, isolated five fractions by precipitation with alcohol followed by ether; the last and most soluble fraction was shown to be N-acetylglucosamine (Fränkel and Kelly 1901a, b). They demonstrated that reaction yielded not only glucosamine/chitosamine, formula $C_6H_{13}NO_5$, and acetic acid but also small amounts of monoacetylglucosamine, formula $C_6H_{15}NO_6$ (Scheme 1.3). This compound was identical with this previously obtained by N-acetylation of D-glucosamine by Breuer (1898). Fränkel and Kelly (1903) concluded that chitin was an acetylated and aminated polysaccharide. A detailed discussion on the hydrolysis of chitin and on the structure of products can be found in the memoir by Bounoure (1919).

1.3.5 Emil Fischer

Emil Fischer (1852–1919, Nobel Prize in 1902) was a German organic chemist and also a pioneering figure in biochemistry (Freudenberg 1967; Lichtenthaler 2002). While working at the University of Munich in the early 1880s, Fischer found that phenylhydrazine converted sugars into osazones whose crystals had characteristic forms that could be identified. The chemistry of phenylhydrazine

Scheme 1.3 Structure of the acetylglucosamine proposed by Fränkel and Kelly (1901a)



was accidentally discovered by him in 1884 when he worked in Strassburg (Fischer 1884). Fisher also proposed the synthesis of glucosamine and he has written upon the value of this discovery: “The synthesis of glucosamine showed it to be an intermediate grape sugar and the α -amino acids, so providing one of the longest sought-for bridges between the carbohydrates and the proteins” (Fischer 1912; Bunge 1912).

At the beginning of 1900s, Fischer and its Ph.D. student Hermann Leuchs proposed the synthesis of glucosamine and established its constitution (Fischer and Leuchs 1902, 1903). By the combination of D-arabinose and ammonium cyanide, or D-arabinose-imine with hydrogen cyanide, D-glucosaminic acid was obtained and its lactone reduced to glucosamine. Glucosamine formed a penta-acetyl derivative and also an oxime, semi-carbazone and phenyl hydrazine. However, it could not be converted into glucose, though it gave glucose phenyl osazone when heated with phenyl hydrazine (Fischer and Leuchs 1903). Nitrous acid converted it into a new compound regarded as a sugar, and also termed chitose ($C_6H_{10}O_5$). This formed chitonic acid when oxidized. Glucosamine was then regarded as a derivative of chitose, and termed chitosamine, confirming the conclusions previously reported by Fränkel and Kelly (1901a, b). In another contribution, chitose was shown by Fischer and Andreae (1903) to be a hydrated furfuran derivative rather than a true sugar, formed by simultaneous elimination of the amino group and anhydride formation.

1.3.6 James Irvine

James Colquhoun Irvine (1877–1952) was a Scottish organic chemist, Professor of Chemistry at the University of St Andrews. Irvine studied chemistry at the University of Leipzig under the supervision of Friedrich W. Ostwald (1853–1932, Nobel Prize in chemistry in 1909). Irvine was appointed Professor of Chemistry in 1909 at the University of St Andrews. Irvine (1909) first reported the specific rotatory power of chitin in hydrochloric acid solution ($^{20}[\alpha]_D - 14.1^\circ$) and its index of refraction (1.525). He also studied the action of acetyl-bromide on glucosamine hydrochloride with the object to clarify the constitution of structural unit of chitin. Although the theoretical amount of nitrogen was evolved when glucosamine was decomposed by nitrous acid, the product of the change was not a simple hexose, but the hydrated furan derivative known as chitose. Chitose was formed under all conditions when nitrous acid acted on glucosamine, and this accounted for the alternative name chitosamine (Irvine 1909; Irvine et al. 1911). Pursuing his work on chitin derivatization into aceto-halogen derivatives, Irvine with his pupil Alexander Hynd concluded that glucosamine may be derived from either glucose (Irvine and Hynd 1912) or mannose (Irvine and Hynd 1914) according to the method of preparation. Glucosamine as an α -amino-derivative had the D-glucose configuration. Their conclusions were in agreement with those published by Fischer and Leuchs (1903) and by Hamlin (1911). However, Irvine and Hynd stated that no rigorous proof could be offered in support of this latter assumption and “the

displacement of any group by any other group may be accompanied by a Walden inversion". The stereochemical arrangement of the amino-group was thus uncertain.

Chitosamine, 2-amino-2-deoxy-D-glucose, was then considered as an aminohexose from chitin, and alternatively described as glucosamine or mannosamine. However, there was a period of confusion for the nomenclature related to this designation until the work of Karrer.

1.3.7 Paul Karrer

In the early 1920s, the Swiss chemist Paul Karrer (1889–1971), Nobel Prize winner in 1937 for his work on vitamins (the prize was shared with British chemist Norman Haworth), published several studies on the chemistry and biochemistry of chitin (Karrer and Smirnof [1922](#); Karrer and Hofmann [1929](#); Karrer and von François [1929](#); Karrer et al. [1924](#); Karrer [1930](#); Karrer and White [1930](#)). Karrer studied chemistry at the University of Zurich with Alfred Werner (Nobel Prize in chemistry in 1913) and obtained his thesis in 1911. After a position of researcher at the Georg-Speyer Haus Foundation in Frankfurt working with Paul Ehrlich (Nobel Prize in medicine in 1908), Karrer in 1919 accepted the position of Professor of Organic Chemistry at the *Chemisches Institut* of Zürich and succeeded Werner as director of this institute (Dahn et al. [1969](#); Eugster [1972](#); Roche [1972](#); Wettstein [1972](#); Beer [1977](#)). Karrer started studies on the chemistry of sugars and polysaccharides such as starches, dextrans, glycogen, inulin, cellulose, chitin, etc.

Karrer studied the degradation, chemistry and biochemistry of chitin and he also prepared different derivatives such as glucosamine or chitosamine and N-acetylglucosamine (Karrer and Smirnof [1922](#); Karrer et al. [1924](#)). The powerful acid hydrolysis of chitin led to the removal of the acetyl group from the macromolecule, giving thereby both glucosamine and acetic acid, confirming previous papers on this topic. Karrer demonstrated that complete acid hydrolysis of chitin yielded D-glucosamine and acetic acid in nearly theoretical amounts if it was assumed that polysaccharide was composed only of mono-acetyl-D-glucosamine units (Karrer and Smirnof [1922](#); Karrer et al. [1924](#)), in agreement with the result of Brach and von Fürth ([1912](#)). Later, Zechmeister and Tóth ([1931](#)), and Bierry et al. ([1939](#)), using chemical procedures and enzymatic reactions, reported a similar conclusion.

In 1929, Karrer and his student Hofmann reported the formation of N-acetylglucosamine (in 50% yield) by the action of the gut contents of the edible snail *Helix pomatia* on lobster chitin (Karrer and Hofmann [1929](#)). The same year, Karrer and another pupil, Götz von François, reported the isolation of 80% of the theoretical amount of acetylglucosamine from an enzymatic digest of a fungal chitin from *Boletus edulis* (Karrer and von François [1929](#)). Karrer's group also showed that re-acetylated chitosan was hydrolyzed by chitinases with the produc-

tion of acetylglucosamine. Chitosan re-acetylated remained a substrate for *Helix* chitinase while chitosan derivatives containing formyl, propionyl, butyryl and benzoyl groups were not substrates (Karrer and von François 1929; Karrer 1930; Karrer and White 1930).

Karrer (1930) also studied the preparation of chitosan. He pointed out that chitosan gave low nitrogen values in a Kjeldahl analysis due to the hydrolysis of some of the amine groups to hydroxyls during the synthesis of chitosan. Later, this was confirmed by Clark and Smith (1936). For Karrer, chitosan was a mono-acetyl-di-glucosamine and this was demonstrated by the fact that chitinase acting on chitosan gave both N-acetylglucosamine and glucosamine (Karrer 1930). Finally, Karrer prepared several derivatives of chitin. For instance, he suggested that phosphoric esters might be of interest in the metabolism of chitin (Karrer et al. 1943). He also studied their anticoagulant activity.

1.3.8 *Albert Hofmann*

Albert Hofmann (1906–2008) began in 1925 his studies in chemistry at the *Chemisches Institut* and prepared his doctorate under the supervision of Karrer on the elucidation of the sugar building blocks of chitin (Hofmann 1929; Finney and Siegel 2008; Hagenbach et al. 2013). Hofmann presented his dissertation entitled “*Über den enzymatischen abbau des chitins und chitosans*” in the spring of 1929. The same year, after leaving university, Hofmann took a job with Sandoz Laboratories in Basel, where he stayed for more the four decades. His main interest was the chemistry of plants and animals. Hofmann also conducted important research on the chemical structure of chitin.

Hofmann’s thesis described the first enzyme that very efficiently degraded chitin (Hofmann 1929; Karrer and Hofmann 1929; Finney and Siegel 2008), obtained from the crude extracts of a common snail, *Helix pomatia*. Hofmann and Karrer (1929) proposed the name chitinase for the active principle of the gastro-intestinal juice of the vineyard snail used. Its action on partially deacetylated chitin (chitosan) led to the formation of oligosaccharides, resistant to acid hydrolysis, containing N-acetyl groups and forming insoluble sulphates. In 1935, Freudenberg and Eichel have reported that the same chitinase destroyed the blood activity of human urinary mucosubstances and liberated N-acetyl-aminosugar from them (Freudenberg and Eichel 1935). Hofmann and Karrer (1929) conclusively demonstrated that chitin was a polymer of N-acetylglucosamine, and chitosan a polymer containing both glucosamine and N-acetylglucosamine. However, it was only in 1946 that Purchase and Braun (1946) clearly elucidated the chemical structure of chitin using hydrolyzing experiments, and in 1977, Muzzarelli reported its distribution, along with that of its derivative chitosan, in the living species (Muzzarelli 1977).

1.4 Exploration: 1930–1950

At the end of the 1920s, the first X-ray diffraction patterns showed considerable similarity to patterns from cellulose and strengthened but did not prove the case in favor of glucosamine, the structural unit of chitin (Herzog 1924; Gonell 1926; Meyer and Mark 1928). Glucosamine was then the object of numerous fundamental studies (Karrer and Smirnofff 1922; Fränkel and Jellinek 1927; Bergmann and Zervas 1931; Elson and Morgan 1933; Bergmann et al. 1934; Kawabe 1934; Morgan and Elson 1934; Cox et al. 1935; Cutler et al. 1937; Cox and Jeffrey 1939; Cutler and Peat 1939; Bierry et al. 1939; Freudenberg et al. 1942). However, much controversy and debate surrounded the constitution of glucosamine, and in particular the stereochemical position of the amino group (Meyer 1942). Many reactions indicated that the amino group had the same position as the hydroxyl group in mannose, while other reactions suggested a relationship with glucose.

Between 1930 and 1950, X-ray analysis became the most reliable method for the differentiation between chitin and cellulose in cell walls of fungi (Farr and Sisson 1934; Clark 1934; Mark 1943; Frey 1950). During this period, chitin and chitosan attracts considerable attention with the exploration of natural fibers. In the mid-1930s, the first chitosan films and fibers were patented by George W. Rigby (Rigby 1936a, b, c, d, e, 1937). There was also the first use in papermaking industry (Lubs 1937; Larson 1939, 1940), textile (Heckert 1937; Arnold 1939), photography (Marasco 1938, 1939; White 1944), and as adhesives (Maxwell 1939). The names that marked this period were Kurt H. Meyer, Max Bergmann, László Zechmeister, Norman Haworth, and Albert G. Richards.

1.4.1 Kurt H. Meyer

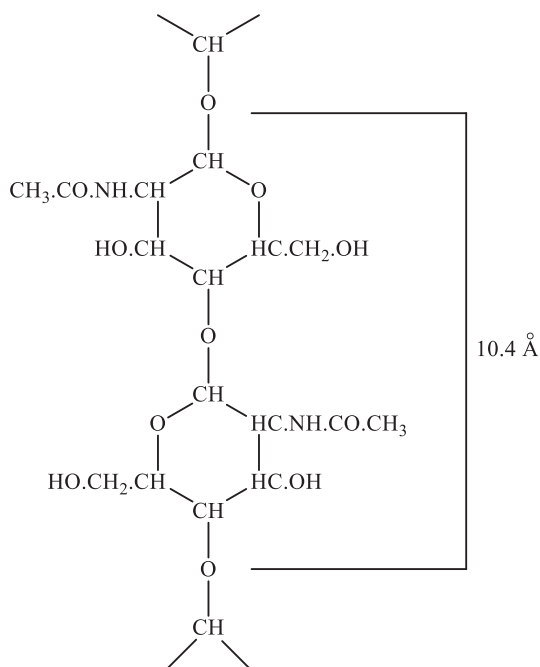
Kurt Heinrich Meyer (1883–1952) was a German chemist of Baltic origin (born in Dorpat). Meyer studied chemistry in Leipzig. After obtaining his Ph.D., he traveled with his father (a famous pharmacologist) through several continents, and spent a year in Rutherford's laboratory in England. After his return to Germany, Meyer went to Bayer's laboratory. After the war, he returned to Munich as Professor in Chemistry until 1920, when he became head of a research laboratory of the I.G. Farben Company. In 1932, Meyer accepted a position at the University of Geneva and became Director of the Organic Chemical Institute (Mark 1952; Jeanloz 1956). Meyer published several important contributions on natural and synthetic polymers and their chemistry. Many of them were devoted to chitin (Meyer and Mark 1928; Meyer and Pankow 1935; Meyer and Wehrli 1937; Meyer 1942, 1950).

In 1924, Herzog was the first to show the crystalline nature of chitin using X-ray diffraction Herzog (1924). The crystalline nature was confirmed and amplified 2 years later by Gonell, under the supervision of Herzog, who arrived at a hexagonal unit cell (Gonell 1926). Indeed, Gonell, studying X-ray data of chitin (*Goliathus*

giganteus), proposed and discussed a rhombic cell with the dimensions $a = 19.42 \text{ \AA}$, $b = 10.40 \text{ \AA}$, and $c = 11.58 \text{ \AA}$ (the cell contained 10 acetylglucosamine units). However, Gonell eliminated it because such a cell could not contain the ten sugar units which were indicated by density measurements (Gonell 1926). He preferred a hexagonal unit cell with 18 glucosamine units. His work was comprehensively discussed by Professor Meyer.

Meyer and Mark (1928), aware of the biological analogy between chitin and cellulose and of the similarity of their structure, proposed a rhombic unit cell. The analogy between the two polysaccharides was apparent in the main features of the X-ray diagram. This analogy led Meyer and Mark to the conclusion that the units of glucosamine were united in the same manner as the glucose units in cellulose, that was, in 1,4- β linkage, each chain having a diagonal screw axis. The authors ascribed to chitin the constitution shown in Scheme 1.4 and assumed that chitin had a micellar structure of parallel oriented chains similar to that cellulose. At the beginning of the 1930s, this structure received important support when Bergman isolated chitobiose, a product of acetolysis (Bergmann et al. 1931a, b, c). The assumption that chitin had a micellar structure of parallel oriented chains similar to that of cellulose was also confirmed in the same period by the demonstration of presence of β -glucosidic linkages by Zechmeister using incomplete acidic hydrolysis (Zechmeister et al. 1932; Zechmeister and Tóth 1933). However, in the mid-1930s, Schorigin stated that the unit of the structure was neither glucose nor mannose, but another sugar (named chitose), differing in configuration (Schorigin and Hait 1934, 1935; Schorigin and Makarowa-Semljanskaja 1935a, b).

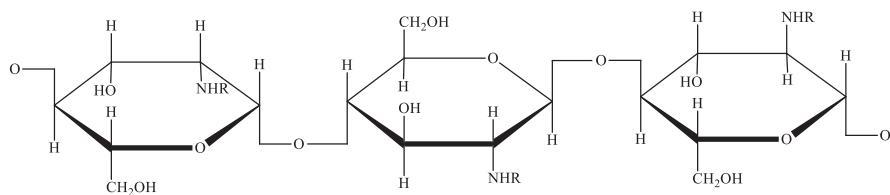
Scheme 1.4 Structural formula of chitin proposed by Meyer and Mark (1928) in agreement with that suggested by Löwy (1909)



A detailed analysis by Meyer and Pankow (1935) provided the basis for the structure commonly accepted in the 1950s (Meyer 1950; Whistler and Smart 1953). This analysis was made on a tendon of the rock lobster *Palinurus vulgaris*, and led to a rhombic cell comprising four chitobiose units with the dimensions $a = 9.40 \text{ \AA}$, $b = 10.46 \text{ \AA}$ (fiber axis), and $c = 19.25 \text{ \AA}$. Indeed, the cell contained 8 acetylglucosamine units (7.9 calculated from the density = 1.415). The analogy with cellulose was apparent in the main features of the diagram proposed for the unit cell of chitin: equal numbers of chains were arranged in opposing directions, the rings followed one another in a diagonal screw sequence and all rings lay flat in one plane. These results have been confirmed by Clark and Smith (1936) but stated the dimension of the a axis as 9.25 \AA . Later, Lotmar and Picken (1950) using chitin from the same source, i.e. *Palinurus vulgaris*, confirmed the a and c values but preferred 10.27 \AA for b . Similar figures were also obtained using fungal and insect chitin. Heyn (1936a, b) stated dimensions of $a = 9.70$, $b = 10.4$, $c = 4.6 \text{ \AA}$ for fungal chitin and the diagrams obtained were almost the same as those from control chitin from cockroaches. Similar X-ray patterns were obtained from chitin in plant cell walls (Khouvine 1932; Heyn 1936c; van Iterson et al. 1936).

Meyer and Pankow (1935) also indicated that acetyl groups alternated from one side of the chain to the other on passing from one residue to the next (evidence in favor of β -linking) and that while chains along the a axis ran in the same direction, the direction was reversed in their neighbors in the direction of the c axis. This alternation in the direction of contiguous chains was also found in cellulose (Meyer and Wehrli 1937; Meyer 1942). Later, the crystal structure determined by Meyer and Pankow (1935) was slightly modified by Darmon and Rudall (1950), who also suggested that there were $\text{C}=\text{O}\cdots\text{H}-\text{N}$ and $\text{C}=\text{O}\cdots\text{H}-\text{O}$ hydrogen bonds as well as free $\text{O}-\text{H}$ and $\text{N}-\text{H}$ groups in the crystal. Pursuing his studies on chitin chemistry, Meyer reported that solutions of fungal chitin in acids had the same viscosity as those of animal chitin (Meyer and Wehrli 1937). It was noted that the viscosity of solutions of chitin in nitric acid solution was of the same order as that of similar solutions of cellulose indicating a degree of polymerization of more than a hundred. Fikentscher (1932) previously reported similar conclusion.

At the beginning of the 1940s, it was clear from all the results published that animal and plant chitins were essentially similar and that, as was suggested by Meyer, chitin closely resembled cellulose in structure and both consisted of long primary valence chains of glucose residues (Picken 1940; Meyer 1942). The constitution of chitin shown in Scheme 1.5 has been recognized. Later, this structure was



Scheme 1.5 Structural formula of chitin accepted in the 1940s

also identified by enzymatic analysis and infra-red spectroscopy (Darmon and Rudall 1950; Orr 1954; Brock 1957; Spedding 1964).

1.4.2 Max Bergmann

At the end of the 1920s, there seemed little doubt that nearly all of a purified chitin can be regarded as a polymeric form of N-acetyl-2-amino-2-deoxy-D-glucose (Tracey 1957; Foster and Webber 1961; Kent 1964). However, it remained to determine the mode of linkage. On this topic, Bergmann published several papers (Bergmann and Zervas 1931; Bergmann et al. 1931a, b, c). Bergman was also the first to suggest that glucosamine had the typical pyranose ring structure (Bergman et al. 1934). In the proposed structure, the six atoms of the ring were nearly coplanar, the oxygen atom being slightly displaced out of the plane of the carbon atoms.

Max Bergmann (1886–1944), a German biochemist, received his Ph.D. in 1911 and became the assistant to Emil Fischer at the University of Berlin. After Fischer's death in 1919, Bergmann automatically became his scientific executor, assuming responsibility for the completion and publication of unfinished researches (Helferich 1969; Katsoyannis 1973; Lichtenthaler 2002). In 1920, he habilitated at the University of Berlin and was appointed to head the newly established *Kaiser-Wilhelm Institut für Lederforschung* in Dresden. In 1933, he left Germany for the United States and he accepted a position of researcher at Rockefeller University in New York City. Bergmann is considered a pioneer of applied sciences and of molecular biology.

With his pupil Leonidas Zervas (Katsoyannis 1973), Bergmann in 1931 was the first to isolate a crystalline disaccharide, as the octa-acetate, by acetolysis of chitin (Bergmann et al. 1931a). He assigned it the name chitobiose. The reducing disaccharide chitobiose was the first low molecular weight polymers obtained from chitin. The product contained two N-acetyl and six O-acetyl groups. Further acetylation confirmed the presence of six hydroxyl groups. Its configuration was identical with the cellobiose molecule except for the substitution of acetylamine groups for the hydroxyl group on carbon-2. The reactions of chitobiose octa-acetate (i.e. oxidation with iodine in alkaline conditions followed by hydrolysis) led to the conclusion that a 1,4-linkage was likely and X-ray evidence and changes of rotation ($^{20}[\alpha]_D -14^\circ \rightarrow +56^\circ$) on hydrolysis indicated a β -form, in agreement with the results published by Meyer and Mark (1928) and by Karrer (Karrer and Hofmann 1929; Karrer and von François 1929). All the results clearly demonstrated that carbon-1 as well as carbon-2 of the reducing moiety cannot be involved in the glycosidic linkage. Shortly thereafter, the postulated β -glycosidic linkage was also confirmed by Zechmeister (Zechmeister et al. 1932; Zechmeister and Tóth 1933).

1.4.3 *László Zechmeister*

László Zechmeister (1890–1972) was born in the city of Győr, in Hungary. In 1907, he commenced his studies in chemistry at the *Eidgenössische Technische Hochschule* in Zurich, under the guidance of Richard Willstätter (1872–1942, Nobel Prize in chemistry in 1927). Zechmeister received his degree as technical chemist in 1911 and then worked at the Kaiser Wilhelm Institute for Chemistry in Berlin-Dahlem from 1912 to 1914. During this period, Zechmeister received his doctorate on “*Zur Kenntnis der cellulose und des lignins*”. After the World War I, in 1921, Zechmeister accepted a position as instructor at the Royal Danish Agriculture and Veterinary Academy in Copenhagen until 1923. Then, he became Professor of Medical Chemistry at the University of Pécs, Hungary (he was only 33 years old). Zechmeister is considered as a pioneer of chromatography (Ettre 1989; Wirth 2013). He published a series of publications on chitin chemistry (Zechmeister and Tóth 1931, 1932, 1933, 1934, 1939a, b, c; Tóth and Zechmeister 1939; Zechmeister et al. 1932, 1939a, b).

In 1931, chitobiose, disaccharide found by Bergmann (Bergmann et al. 1931a) was also isolated by Zechmeister and his colleague Géza Tóth from the products of partial acid hydrolysis of chitin (Zechmeister and Tóth 1931). Chitobiose, originally isolated from lobster shells, was isolated from beetles, snail radulae, and also fungi (Zechmeister and Tóth 1934; Tóth and Zechmeister 1939). In addition to chitobiose, partial degradation of chitin also yielded a trisaccharide chitotriose from the acetolysis mixture, which had been characterized as its crystalline acetate, chitotriose undeca-acetate (Zechmeister and Tóth 1932). A more detailed account about the D-glucosamine or chitosamine was then provided. The authors, using X-ray diffraction measurements and rotary change experiments (Zechmeister and Tóth 1932, 1933), also suggested the β -linkage, in agreement with studies published by Karrer (Karrer and Hofmann 1929; Karrer and von François 1929) and Bergmann et al. (1931a). Zechmeister and Tóth (1934) comprehensively discussed the occurrence of chitin in plants and animals, its characteristics and analysis. A few years later, using enzymic studies, Zechmeister also confirmed the β -linkage (Zechmeister and Tóth 1939a, b, c; Zechmeister et al. 1939a, b).

1.4.4 *Norman Haworth*

Walter Norman Haworth (1883–1950) was a British chemist at the University of Birmingham, known for his work on ascorbic acid and the development of the Haworth projection used in organic chemistry to characterize sugar structures. He studied organic chemistry at the University of Göttingen earning his Ph.D. in Otto Wallach's laboratory. Haworth received the Nobel Prize in chemistry in 1937, shared with Karrer, for his work on carbohydrates and vitamin C.

In 1939, Haworth, Lake and Peat proposed a synthetic method which distinguished between the alternative configurations, glucose or mannose, for glucosamine (chitosamine). The fission by alkali such as sodium methoxide of the ethylene oxide ring in anhydro-sugars leads to the formation of two isomeric sugars, consequent upon the independent rupture of the two bonds of the oxide oxygen atom. In each case, ring opening was accompanied by a Walden inversion at the carbon of the epoxide ring at which the attack occurred and to which the amine group therefore became attached. The method has been to prepare a dimethyl 2,3-anhydro-methylmannoside which has been shown to give rise, with sodium methoxide or sodium hydroxide, to both a glucose derivative and an altrose derivative. The opening of the anhydro-ring in the dimethyl 2,3-anhydro-methylmannoside was then carried out by the agency of ammonia, which gave rise to a derivative of 3-amino-altrose on the one hand and of 2-amino-glucose on the other. The latter was shown to be identical with chitosamine, which might be considered configurationally to be glucosamine and therefore related to the parent sugar glucose (Haworth et al. 1939). It should be pointed out that two other groups came to similar conclusions the same year (Neuberger and Pitt Rivers 1939; Cox and Jeffrey 1939).

1.4.5 *Albert Glenn Richards*

At the end of the 1940s, the works of Albert Glenn Richards, an American Professor of Zoology at the University of Minnesota, on the biochemistry of chitin were acknowledged to have made an important contribution (Richards 1947a, b, 1949, 1951, 1952, 1958; Richards and Anderson 1942; Richards and Cutkomp 1946; Richards and Korda 1948; Richards and Pipa 1958). From 1890 to 1930, the most extensive work on chitin in fungi has been done with the genera of *Aspergillus*, *Agaricus*, *Boletus*, and *Phycomyces*. The first and complete list of forms tested in the fungi was only reported in 1951 by Richards in his book on the integument of the arthropods, which really set the benchmark for chitin zoological research (Richards 1951). This monograph is considered as a fundamental guide to the voluminous literature on the subject. Richards also reviewed some histochemical methods for the detection of chitin. Another fundamental book on the biology of the arthropod cuticle was published in 1975 by Anthony C. Neville (Neville 1975).

1.5 The Period of Doubt: 1950–1970

1.5.1 Literature Review

During the period of exploration (1930–1950), chitin and chitosan attracted considerable attention with the exploration of natural fibers and the first applications but lack of adequate manufacturing facilities and mostly cutthroat competition from synthetic polymers restricted their commercial development. From 1950 to 1970, chitin and chitosan entered in a period of doubt although much progress has also been made on their isolation, production and fundamentals (Wigglesworth 1948, 1957; Meyer 1950).

The first books on chitin were the well-known monographs on the integument of the arthropods by Richards edited in 1951 (Richards 1951) and on the biochemistry of aminosugars edited in 1955 by Paul Welberry Kent and Michael Wellesley Whitehouse (Kent and Whitehouse 1955). A few years later, other comprehensive books were published by Peter Bernfeld on the biogenesis of chitin (Bernfeld 1963), by Elwyn T. Reese on a discussion of the advances in the enzymic hydrolysis of natural substances (Reese 1963), and by Charles Jeuniaux on chitin and its enzymatic breakdown (Jeuniaux 1963).

In 1953, Roy Lester Whistler and Charles Louis Smart wrote a short chapter on chitin chemistry in their famous book “Polysaccharide Chemistry” (Whistler and Smart 1953). In 1955, M.V. Tracey published a review on the detection and determination of chitin in plant materials, and the quantitative analytical methods (Tracey 1955). Two years later, Tracey also published a detailed review entitled “chitin” (Tracey 1957). In 1958, Allan B. Foster and Maurice Stacey published a detailed chapter on the aminosugars and chitin in *Encyclopedia of Plant Physiology* (Foster and Stacey 1958). Biological aspects of chitin were summarized in general reviews by Richards (1958), Picken (1960), Jeuniaux (1963), and K.M. Rudall (1963), chemical aspects by Foster and Webber (1961), and its production by James N. BeMiller (BeMiller 1965). Later, Rudall also addressed the concept of the chitin-protein. He detailed the chitin-protein complexes and their conformation in two comprehensive reviews (Rudall 1967, 1969).

In 1964, Paul W. Kent published a comprehensive review on chitin and mucosubstances (Kent 1964). The same year, John S. Brimacombe and J.M. Webber discussed the chemical structure, distribution and isolation of mucopolysaccharides (Brimacombe and Webber 1964). The progress on chitin orientation in cuticle and its control were summarized by Neville (1967). Three chapters by Friedman (1970), Honke and Scheer (1970), and Jeuniaux (1971) were devoted to the zoological importance of chitin and its role in biochemical evolution. The first interdisciplinary book on chitin was published in 1973 (Muzzarelli 1973).

1.5.2 Selected Highlights

From this period of doubt (1950–1970), I choose to highlight the following studies. Until the beginning of the 1950s, the methods for isolating pure chitin by decalcification with acids and deproteinization with alkalis proposed in the nineteenth century by Odier, Children, Gilson and Winterstein, and later by other colleagues such as Scholl have continued virtually unchanged (Kent 1964). Between 1950 and 1960, “new” methods for the isolation of chitin were proposed by Blumberg (Blumberg et al. 1951), Hackman (Hackman 1953a, b, 1954, 1959, 1960, 1962; Foster and Hackman 1957), Roseman (Horowitz et al. 1957; Blumenthal and Roseman 1957), BeMiller (1965), and Broussignac (1968). Methods for the isolation of a series of oligosaccharides (Barker et al. 1957, 1958) and for the production of chitosan (Wolfrom 1958; Wolfrom et al. 1958; Wolfrom and Han 1959; Horowitz et al. 1957; Broussignac 1968) from chitin have also been proposed. All these methods were listed by Muzzarelli (1973).

In 1950, Jeanloz, studying periodate oxidation of chitin and chitosan, demonstrated that only 1-4-linkages were present in these polysaccharides (Jeanloz 1950; Jeanloz and Forchielli 1950, 1951). The same year, Darmon and Rudall (1950), studying the infra-red spectra of chitin, were able to identify the principal bands associated with the amido and hydroxyl groups vibrations and observed their dichroism. The authors correlated the infra-red absorption spectrum with the X-ray data in order to give a detailed structure for chitin. Darmon and Rudall (1950) also studied the infra-red spectra of deacetylated chitin and chitin nitrate.

In 1958, Giles and co-workers reported that chitin was a polymer of the amino sugar acetylglucosamine (Giles et al. 1958). However, in a small percentage of residues the acetyl group may be missing, leaving glucosamine, so that the chain as a whole may be positively charged. This could be important in chitin-protein cross-linking (Hackman 1960) and as one of the factors involved in orientation of the chitin chains (Attwood and Zola 1967; Neville 1967). At the beginning of the 1960s, it is evident that chitin, considered exclusively as a homopolysaccharide, was not normally found, and *in situ* it was associated with other substances, notably proteins, by hydrogen bonds and covalent linkages (Whistler and Smart 1953; Rudall 1963, 1965; Kent 1964). A convenient method of distinction proposed by Hackman (1960) reserved the name chitin for the chemically purified material and native chitin for the complex in which it is involved in tissues. The chemical structure of chitin was agreed to be that of a long unbranched polysaccharide in which N-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucopyranose) residues were linked in the β -(1 \rightarrow 4) positions. At the end of the 1960s, it was accepted that three different molecular systems occurred as the chief skeletal support of living organisms, namely the cellulose systems present in plants, the collagenous systems present in animals, and the chitinous system (Rudall 1965, 1967, 1969; Rudall and Kenchington 1973). Chitin occurred as an alternative to cellulose in plants and as an alternative to collagen in animals. Significant progress was made in the chemistry and the production of chitin (Brimacombe and Webber 1964; Conrad 1966).

Although initiated in 1920s, an important contribution was made on the structure of “purified” chitin and its polymorphism during the period 1950–1970. Indeed this was the subject of hot debate between the different laboratories (Kreger 1954; Rudall 1955). X-ray fiber diagrams of oriented chitin samples finally showed an obvious similarity to those of cellulose. Comparison of the X-ray data for chitin from different sources had also revealed the existence in nature of more than one polymorphic form, namely α -chitin (Meyer and Mark 1928), β -chitin (Lotmar and Picken 1950) and γ -chitin (Rudall 1963). The structure of α -chitin has been investigated more extensively than that of either the β - or γ -form because it was the more common polymorphic form. The three forms have been found in different parts of the same organisms, suggesting that these forms were relevant to the different functions and not to animal grouping. However, it was difficult to assign a physiological role to these crystalline forms.

As already mentioned, the earliest X-ray investigation was that of Gonell (1926) whose results formed the basis for the discussion of Meyer and Mark (1928) on α -chitin. In this paper, a structure analogous to that of cellulose was first proposed. They established the unit cell as orthorhombic. The crystal structure of α -chitin was confirmed by Meyer and Pankow (1935) and by Lotmar and Picken (1950). Its structure was then modified by Darmon and Rudall (1950). Later, the first detailed structure analysis was that of Carlström (1957) who concurred with an orthorhombic unit cell but obtained different dimensions (Table 1.1). Dweltz (1960, 1961) also proposed a structure for α -chitin using X-ray data. The unit cell contained two polysaccharide chains running in opposite directions and four asymmetric N-acetylglucosamine units. This structure was in agreement with infra-red absorption data.

Evidence for the existence of a second crystalline form of chitin was first obtained by Lotmar and Picken (1950). These authors observed a new X-ray pattern for

Table 1.1 Unit cell dimensions (Å) for α -chitin, β -chitin and γ -chitin

Chitin	a	b	c	References
α	11.58	10.4	19.42	Gonell (1926)
α	9.40	10.46	19.25	Meyer and Pankow (1935)
α	9.25	10.46	19.25	Clark and Smith (1936)
α	9.40	10.26	19.25	Lotmar and Picken (1950)
α	4.76	10.28	18.85	Carlström (1957)
α	4.69	10.43	19.13	Dweltz (1960)
α	4.74 ± 0.01	10.32 ± 0.02	18.86 ± 0.01	Minke and Blackwell (1978)
α	4.71	10.30	18.78	Sikorski et al. (2009)
β	4.7	10.3	10.5	Dweltz (1961)
β	4.85	10.38	9.26	Blackwell et al. (1967)
β (anhydrous)	4.85	10.38	9.26	Blackwell (1969)
β (monohydrate)	4.8	10.4	10.5	Blackwell (1969)
β (dehydrate)	4.8	10.4	11.1	Blackwell (1969)
γ	4.7	10.3	28.4	Walton and Blackwell (1973)

deproteinized pens from the squid *Loligo*. This type form which apparently had a unit cell of dimensions $a = 9.32 \text{ \AA}$, $b = 10.17 \text{ \AA}$ and $c = 22.15 \text{ \AA}$, was named β -chitin to distinguish it from the much more common α -chitin. It was found in annelid chaetae (*Aphrodite aculeate*), in the brachiopod *Lingula* and in the skeletal pen of squids (*Loligo*). In arthropods and fungi, only α -chitin appeared to occur. Lotmar and Picken (1950) also noted that the extra-X-ray reflections in the pattern of intact *Aphrodite chaetae* and other tanned material, and suggested that they may be due to ordered protein. Fraenkel and Rudall (1940), studying the X-ray characterization of chitin-protein complexes, previously pointed out the differences between the fiber diagrams of intact and deproteinized insect cuticles. They suggested that the differences were due to modification of the chitin structure due to the presence of complexing protein. Later, Rudall showed that the X-ray patterns of the intact complexes gave many more layer lines than the purified chitin component (Rudall 1955, 1963, 1967). Rudall first remarked that β -chitin was found associated with collagen whereas α -chitin occurred alone or in association with a non-collagenous protein such as arthropodin (Rudall 1955; Richards 1958). He advanced reasons for supposing that the production of α -chitin and collagen may be mutually exclusive. Differences between the two forms of chitin were evidently slight β -chitin being converted to the α -form by dissolving it in anhydrous formic acid or by treatment with strong nitric acid.

Detailed crystallographic investigations have also been reported for the α - and β -forms of chitin by Dweltz (Dweltz 1960, 1961; Dweltz and Anand 1961). Dweltz proposed new crystals structures and comprehensively discussed the spatial configuration of the polymer chain. In both cases, the structures were based upon backbones consisting of straight polysaccharide chains. Basic to the proposed structure for the two systems was the presence of sheets of parallel chains linked by C=O---H-N hydrogen bonds through the amide groups. The forms differed in the sense of the chains in successive sheets. In β -chitin the sheets were all arranged in a parallel manner whereas in the α -form successive sheets were antiparallel. For β -chitin, obtained from the conversion of α -chitin by treatment with formic acid or fuming nitric acid using the experimental protocol published by Lotmar and Picken (1950) and Rudall (1955), Dweltz reported the unit cell to be approximately half that of α -chitin (Table 1.1). For Dweltz, β -chitin must be considered to be a monohydrate in the dry state with the chemical formula $[\text{C}_8\text{H}_{13}\text{O}_5\text{N} \cdot \text{H}_2\text{O}]_n$ and the sugar (glucosamine) in β -chitin was the same as that in α -chitin.

In 1962, Carlström has criticized the structures of α - and β -chitin proposed by Dweltz (Carlström 1962). The main criticism was that the polysaccharide chain configuration was stereochemically unsatisfactory. A straight-chain configuration consisting of glucose units linked together by 1,4- β -glucosidic bonds was sterically impossible. First, Carlström, using the optical transform method, arrived at a new structure for α -chitin (Carlström 1957). An orthorhombic unit cell with $a = 4.76 \text{ \AA}$, $b = 10.28 \text{ \AA}$ (fibre axis), and $c = 18.85 \text{ \AA}$ was proposed. There were two chitobiose units per unit cell. The repeating period along the fibre axis was the same as that of cellulose. Carlström showed that the chain was in the bent form, similar to that proposed for cellulose (Carlström 1962). This was a major difference in the structure

compared with model proposed by Meyer (Meyer and Mark 1928; Meyer and Pankow 1935). The $-\text{NHCOCH}_3$ groups were also assumed to be planar and to be predominantly perpendicular to the fibre axis. An intramolecular hydrogen bond was formed between the carbon-3 hydroxyl group and the ring oxygen of the next acetylglucosamine residue (Carlström 1957, 1962). Carlström proposed a scheme of full intermolecular hydrogen bonding of the $\text{C}=\text{O}\cdots\text{H}-\text{N}$ groups along the direction of the a axis. He noted that the hydroxyl group attached to carbon-6 was found to have some rotational freedom. Each glucose residue had a distance between the connecting oxygens of about 5.45 \AA , and this was supported by the excellent crystal structure determination of cellobiose. Carlström (1962) finally reported that optically derived Fourier transforms based on his proposed structure had intensity distributions similar to the observed X-ray intensities.

The same year, Carlström's structure was partially confirmed by Ramachandran and Ramakrishnan (1962). These authors suggested that the data obtained by Dweltz were re-estimated due to differences in the observed intensities. However, they concluded that "it was not possible to say that one structure was superior to the other". The complete intermolecular $\text{C}=\text{O}\cdots\text{H}-\text{N}$ hydrogen bonding scheme, originally proposed by Carlström from X-ray data, was also in agreement with the infra-red studies published by Pearson et al. (1960). These authors also reported that there were no free OH and NH groups nor any $\text{C}=\text{O}\cdots\text{H}-\text{O}$ bonds in the chitin crystal. With the aid of a scale model of the chitin unit cell, a number of hydrogen-bonding schemes involving the primary hydroxyl groups were proposed. Several attempts were also made over the following 20 years to improve on Carlström's model (Bouligand 1965; Blackwell 1969; Blackwell et al. 1965, 1967, 1980; Neville and Luke 1969a, b; Neville 1970; Ramakrishnan and Prasad 1972; Gardner and Blackwell 1975; Minke and Blackwell 1978).

Another milestone in the discovery of chitin's structure and arrangement was made by Bouligand in 1965 through extensive ultra-structures analyses of crustacean cuticles (Bouligand 1965; Berezina 2016). Bouligand discovered that chitin adopted a stereotypic arrangement (helicoid structure) in arthropods. Thus, three types were also found: α -, β - and γ -chitin. In 1969, Neville and Luke also found that chitin in insect cuticles adopted the Bouligand arrangement as well (Neville and Luke 1969a, b). They suggested that cuticle was arranged in non-lamellate and lamellate systems (Neville and Luke 1969a, b; Neville 1970). The original discovery of cuticle deposition by Bouligand was extensively reviewed and discussed by Neville in the mid-1970s (Neville 1975; Neville et al. 1976).

Blackwell and co-workers reinvestigated the structure of chitin (Blackwell 1969; Blackwell et al. 1965, 1967; Gardner and Blackwell 1975). These authors, studying the crystalline forms of the chitin, reported that they differed in the packing and polarity of the adjacent chain. α -Chitin was the most abundant form and it was found in certain fungi and in arthropod cuticles. α -Chitin was the tightly compacted, most crystalline polymorphic form where the chains were arranged in anti-parallel orientation. Sheets of chains were arranged in stacks along the a axis, the sheets being linked by $\text{C}=\text{O}\cdots\text{H}-\text{N}$ hydrogen bonds approximately parallel to the a axis. The strong inter- and intra-molecular bonding leads to the formation of long micro-

fibrils. Blackwell and co-workers also pointed out a number of deficiencies in the model proposed by Carlström (Minke and Blackwell 1978). For α -chitin, their two main criticisms were the following: 1) the CH_2OH side chains were not hydrogen-bonded although infra-red spectroscopic studies showed that all the hydroxyl groups formed donor hydrogen bonds and 2) the presence of two amide I peaks suggested that the arrangement of the amide groups cannot be correct. Marchessault and Sarko (1967) previously reported similar conclusions.

The structure of β -chitin from pogonophore tubes and from the spines of marine diatoms (*Thalassiosira fluviatilis*, *Cyclotella cryptica*) has been refined by rigid-body least-squares methods by Blackwell's group. The β -chitin was the form where the chains were parallel. The unit cell was monoclinic, in agreement with Dweltz' model but different cell dimensions were found (Table 1.1). The increase in the values along the a axis, compared with that for α -chitin, indicated a greater spacing between the chains in this direction. The structure consisted of an array of poly-N-acetyl-D-glucosamine chains all having the same environment, which were also linked together in sheets by $\text{C}=\text{O}\cdots\text{H}-\text{N}$ hydrogen bonding of the amide groups. The structure proposed were consistent with the swelling properties of β -chitin and can be seen to be analogous to that of native cellulose (Gardner and Blackwell 1975). Both structures contained extended parallel chains and can be visualized as an array of hydrogen-bonded sheets. β -chitin swelled extensively in water and has been shown to form a series of crystalline hydrates. β -chitin from Polychaetae, when precipitated from acids, also assumed the α -form. In 1988, Blackwell suggested that γ -chitin may be "a distorted version of either α - or β -chitin rather than a true third polymorphic form" (Blackwell 1988).

1.6 The Period of Application: From 1970 Until Now

Despite the significant progress that was made between 1930 and 1970, few researchers at that time believed in the potential that chitin and chitosan had. "Re-discovery" and revived interest in the 1970s encouraged the need to better utilize biowastes from marine crustaceans, due to the introduction of regulation on the dumping of untreated shellfish wastes into the oceans (Muzzarelli 1977; Roberts 1992). From 1970 to 1980, chitin and chitosan entered the period when they reached maturity. Several manufacturers started to produce and to market these products. Chitosan was produced industrially for the first time in Japan in 1971 (Hirano 1989). This industrialization of the production has also contributed enormously to their development. The expansion of the two polysaccharides was also made possible by the first concrete applications in cosmetology, pharmacy, personal care uses, food uses, agriculture, biotechnology, clarification and waste management (sludge dewatering), dentistry and medicine (Feofilova 1984; Zikakis 1984; Hirano 1989; Tsugita 1990; Krajewska 1991; Dodane and Vilivalam 1998). Several patents also claimed the use of chitosan and chitosan derivatives in paper-making (Slagel and Sinkovitz 1973a, b; Plisko et al. 1974).

At the end of 1930s, Kunike was the first to try to produce fibres from chitin (Kunike 1926a, b, c) although the major difficulty was to find a suitable solvent. The discovery of new solvents in the 1970s stimulated new interest in this topic. Research on chitin synthesis, dormant for many years, has also been revived at the end of 1970s due to an unexpected discovery relative to the insecticidal properties of certain benzoylphenyl ureas (Verloop and Ferrell 1977; Hajjar and Casida 1979; Zobelein et al. 1980). This insecticidal action has generated great interest in insect chitin biosynthesis, in particular in order to contribute to a better understanding of the functional organization of the chitin synthase within the integument and its intricate regulation, and to develop environmentally acceptable pesticides (Hackman 1984, 1987; Cohen 1987a). Two steps in arthropod chitin synthesis have been identified, one sensitive to benzoylphenyl ureas and another to tunicamycin. This clearly discriminated chitin formation in animals from that in fungi and yeasts and demonstrated that different targets for interference besides chitin synthase itself can be used successfully (Roberts 1992; Goosen 1997). At the same time, the potent ability of chitin to accelerate wound healing is discovered (Prudden et al. 1970). Subsequently, many works attempted to implement this discovery in many fields. Today, there are more than 2000 applications of chitin, chitosan and their numerous derivatives (Philibert et al. 2017; see other chapters in this book). These biopolymers continue to offer new horizons to scientists and industrials with a wide range of possible modifications and forms.

In 1973, Riccardo A.A. Muzzarelli edited the first interdisciplinary reference book on polysaccharides entitled “Natural chelating polymers” including three chapters on chitin, chitosan and their analytical applications (Muzzarelli 1973). The same year, Walton and Blackwell (1973), and Brimacombe (1973) comprehensively discussed the structural aspects of chitin and its chemistry. In 1977, Muzzarelli edited another famous chitin sourcebook (Muzzarelli 1977). In that same watershed year, the first international conference on chitin and chitosan was held in Boston in April (11th–13th) organized by Vincent LoCicero of the Massachusetts Science and Technology Foundation, and hosted jointly by the MIT Sea Grant Program and the Massachusetts Science and Technology Foundation. Although the conference was hosted by MIT, the Chairman was RAA Muzzarelli of the University of Ancona, Italy. This first symposium was a great success, with participants coming from all over the world (Muzzarelli and Pariser 1978). The 14th and most recent International Chitin and Chitosan Conference organized by the Japanese Society for Chitin and Chitosan was held in Osaka, Japan (August 27–30, 2018).

Numerous patents have been filed since the 1970s, and an abundant scientific literature has built up. Indeed, a large number of generalist reviews, book chapters and books has been published on practically all the aspects of chitin and chitosan, so many that it would not be feasible to cite them all. I chose to highlight the followings: Jeuniaux (1971, 1982), Brimacombe (1973), Muzzarelli (1973, 1977), Rudall and Kenchington (1973), Walton and Blackwell (1973), Whistler (1973), Neville (1975), Sharon (1980), Cohen (1987a, b), Lezica and Quesada Allué (1990), Roberts (1992), Horst et al. (1993), No and Meyers (1995), Winterowd and Sandford

Table 1.2 Selected books on chitin and chitosan published during the last two decades

Year	Title	References
1997	Applications of chitin and chitosan	Goosen (1997)
2001	Chitin: Fulfilling a biomaterials promise	Khor (2001)
2005	Polysaccharides – structural diversity and functional versatility	Dumitriu (2005)
2009	<i>Chitine et chitosane – Du biopolymère à l’application</i>	Crini et al. (2009)
2011	Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications	Kim (2011)
2011	Chitosan: Manufacture, properties, and usage	Davis (2011)
2011	Chitosan for biomaterials	Jayakumar et al. (2011)
2011	Focus on chitosan research	Ferguson and O’Neill (2011)
2012	Chitosan-based hydrogels: Functions and applications	Yao et al. (2012)
2012	Chitosan-based systems for biopharmaceuticals	Sarmento and das Neves (2012)
2014	Chitin and chitosan derivatives. Advances in drug discovery and developments	Kim (2014)
2015	Advances in marine chitin and chitosan	Sashiwa and Harding (2015)
2016	Chitin and chitosan for regenerative medicine	Dutta (2016)
2017	Chitosan based biomaterials. Fundamentals	Amber Jennings and Bumgardner (2017)
2017	Chitosan – derivatives, composites and applications	Ahmed and Ikram (2017)

(1995), Hudson and Smith (1998), Kurita (1998, 2006), Ravi Kumar (2000), Ravi Kumar et al. (2004), Vårum and Smidsrød (2004a, b), Rauh and Dornish (2006), Rinaudo (2006), Crini and Badot (2008), Peniche et al. (2008), Muzzarelli and Muzzarelli (2009), Renault et al. (2009), Kean and Thanou (2011), Nwe et al. (2011, 2014), Sahoo and Nayak (2011), Teng (2012), Wang et al. (2012), Younes and Rinaudo (2015), Gallo et al. (2016), Bonecco et al. (2017), Dima et al. (2017), Nechita (2017), Ahmed et al. (2018), Akbar and Shakeel (2018), Aljohani et al. (2018), Dimassi et al. (2018), Han et al. (2018), Liaqat and Eltem (2018), Nezakati et al. (2018), Pakdel and Peighamardoust (2018), Pellá et al. (2018), Yu et al. (2018), and Zhao et al. (2018). Table 1.2 shows a selection of books on chitin and chitosan published in the last two decades.

1.7 Conclusion

In this chapter, I have divided the history of chitin into five quite distinct periods, each period being illustrated by relevant references that I have chosen to highlight. The first period, from 1799 to 1894, covers its discovery in fungi by Braconnot in 1811 who called it *fongine/fungine*. The name *chitine*/chitin was conferred by

Odier in 1823 “for what has subsequently been found to be the same compound in insects”. The discovery of chitosan, i.e. “*chitine modifiée*”, is attributed to Rouget in 1859 but the name chitosan was introduced in 1894 by Hoppe-Seyler. From 1894 to 1930 came a period of confusion due to the controversy about whether or not chitin was identical with cellulose although as early as 1843 both Lassaigne and Payen had noted that chitin contained nitrogen. The third period, from 1930 to 1950, was marked by the characterization of the structure of chitin, by numerous patents and by the first practical applications. These three different periods are illustrated by considering examples of studies that appeared in the literature. From 1950 to 1970 came a short period of doubt although numerous fundamental studies have been published. Finally, the period of real utilization has been in progress since 1970 and has seen chitin and its main derivative, chitosan, find numerous industrial applications.

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