Chapter IX

Chitinous Structures

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1. Introduction: the concept of chitinous structures

Among the organic molecules used extensively by living organisms in the elaboration of skeletal or cuticular structures, chitin is clearly one of the most important. Chitin has widespread distribution, it is quantitatively abundant in the biosphere, and it occurs in many different kinds of structures.

Since the discovery[1] in 1799 of an organic material in the arthropod cuticle which was particularly resistant to the usual chemical reagents, a material called chitin by Odier[2], the term *chitinous structure* has been commonly used to designate all types of organic structures exhibiting the physical properties of the arthropod cuticle, regardless of chemical composition. Terms such as "pseudochitin" or "chitinoid" were introduced to characterize structures which exhibited only some of the typical properties of true chitin, and entomologists often used the expression "chitinized" to describe the most sclerotized parts of the insect integument.

It is clear that the term *chitinous structure* should be restricted to those formations in which chitin itself is present and plays a structural role. It will be clear from later discussion that chitin does not exist as such in chitinous structures, but is a product of degradation of naturally occurring chitin–protein complexes, for which the term *native chitin* has been proposed[3]. Thus we can define a chitinous structure as a skeletal or cuticular structure which consists of chitin–protein complexes, that is to say glycoproteins or mucoproteins, the prosthetic group of which is entirely or principally chitin.

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The chitin–protein complexes appear to provide the framework which support organic and mineral deposits, and the latter probably confer the special properties of any different type of chitinous structures. Studies on the chemistry and ultrastructure of these highly complex structures have only just begun, although X-ray diffraction work has provided a better knowledge of the chitin–protein framework. This chapter is designed to relate the chemical and architectural organization of the chitin–protein complexes to the general properties of chitinous structures, and to the role these structures play in living organisms and biological evolution.

2. Chemical composition and molecular structure of chitin

Chitin is a linear homoglycan[4] consisting of *N*-acetyl-D-glucosamine units (2-acetamido-2-deoxy-D-glucose), linked by glycosidic bonds in the $\beta(1\text{–}4)$ position. The structural repeating unit is *chitobiose*, the dimer of *N*-acetyl-D-glucosamine. The chemical constitution and properties of chitin have been summarized in this treatise (Vol. 5, p. 208 and pp. 266–270). More exhaustive reviews of chitin chemistry are also available[5–7]. Only certain aspects of chitin chemistry essential to further discussion will be considered here.

(*a*) *Physical properties*

Chitin is a chemically stable constituent of chitinous structures. Chitin is isolated only after destruction or removal of the other constituents. After removing mineral deposits and organic substances by drastic treatments from a typical chitinous structure, for example a lobster shell or an insect cuticle, sheets of "pure" chitin may be obtained. These sheets are colorless, or white in color if thick enough, and bear all the morphological characteristics of the starting shell or cuticle.

However, isolated chitin sheets do not exhibit the remarkable properties of the original chitinous structures, such as hardness, rigidity, or impermeability. Chitin sheets do show a relatively high tensile strength; according to the species, values are reported to be between 10 and 58 kg/mm^2 in the case of dry chitin sheets isolated from arthropod cuticles, and about 2 kg/mm^2 in the case of moist material[8–10]. Other physical properties of isolated chitin differ considerably from those exhibited by the naturally occurring chitinous structures. Sheets of isolated chitin are soft and pliable, and are permeable to water and gases. Clearly, many of the mechanical and physical properties

of chitinous structures are not due to chitin itself, but to chitin complexes and deposits.

(*b*) *Nature of the amino-sugar residues in chitin*

Elucidation of the nature of interactions between chitin molecules and between chitin and other substances depends on precise knowledge of the chemical composition of the polysaccharide. While it is generally assumed that chitin is built only of *N*-acetyl-D-glucosamine units, the nitrogen content of purified chitin has been reported by numerous workers to be 6.1–6.7%, while the calculated value is 6.89%. These discrepancies are generally explained by incomplete purification of the chitin, or by the fact that chitin is a strong adsorbent, sometimes used indeed in chromatography.

Probably more significant are observations that the enzymatic hydrolysis of pure chitin by chitinase (EC 3.2.1.14) plus chitobiase (EC 3.2.1.29) leads to the production of small amounts of free glucosamine along with acetylglucosamine[11,12] or to a triose in which one or two amino groups are not acetylated[12]. The presence of glucosamine is apparently not due to the action of deacetylases, since such enzymes were not detected in the enzymatic extracts used[11,12]. There is a possibility that partial deacetylation occurs during chitin isolation.

These observations led Giles *et al.*[13] to a re-examination of the chemical composition of chitin extracted by mild procedures. The analysis of C, H, and N in different samples of purified and dried lobster chitin suggests that the proportion of amino sugars are 82.5% acetylglucosamine, 12.5% glucosamine, and 5% water. Chitin chains would thus be composed of about 1 glucosamine for 6–7 acetylglucosamine residues, in addition to firmly bound water. This interpretation has been discussed at length by Rudall[14], who concluded on the basis of X-ray-diffraction diagrams, infrared-absorption spectra and density measurements, that chitin may depart from an idealized poly-*N*-acetylglucosamine structure in having one residue deacetylated for every 6 or 7 residues, with bound water replacing missing acetyl groups to maintain density and crystallographic properties consistent with those of a poly-*N*-acetylglucosamine. A similar interpretation is given for the chitin of cuttlefish shell and squid "pen", which is of the crystallographic β-type[12].

(*c*) *Macromolecular structure*

The chain configuration of chitin has been difficult to elucidate, due to its

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insolubility and the possibility of structure modification during isolation. It is obvious however, from the observations of Herzog[15] and Gonell[16] that the structure is crystalline and shows a great similarity to that of cellulose. The studies of Meyer and coworkers[17,18] on crustacean chitin led to the classical figure of an orthorhombic unit cell, having the dimensions $a = 9.40$ Å, $b = 10.46$ Å, and $c = 19.25$ Å (Fig. 1). Chitin fibers are extended along the b-axis, and adjacent chains run in opposite directions. Owing to the β-glycosidic linkage between acetylglucosamine residues, the acetamido groups alternate from one side to the other along the chitin chain.

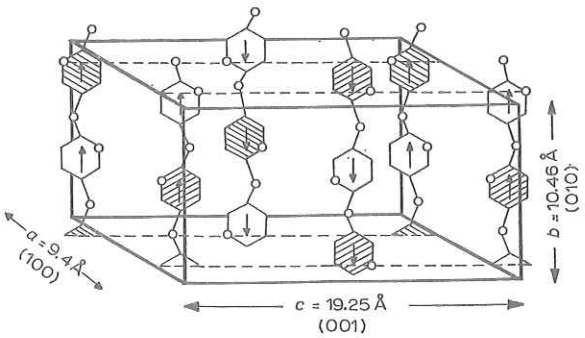
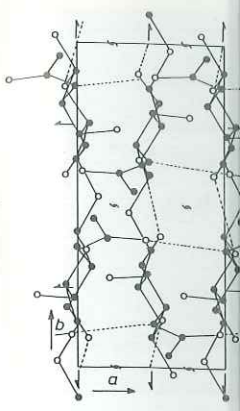


Fig. 1. Unit cell of chitin, after Meyer and Pankow[18]. Arrows indicate the alternating directions of chains.

Using more elaborate methods, Carlström[19] confirmed these general conclusions, but proposed a simpler orthorhombic unit cell, the value of a being only 4.76 Å. This would mean that the chitin unit cell is composed of two chains, antiparallel in direction. The study of infrared absorption spectra permitted the determination of the position of intramolecular and interchain bonding. According to Carlström[19] the adjacent residues of the same chain are not only covalently bonded by β(1–4) glycosidic linkages, but also hydrogen bonded between O_3 and O_5'. The neighbouring chains are linked by hydrogen bonds CO··H·N between adjacent aminoacetyl groups; presumably all the NH groups of a chitin chain are hydrogen-bonded to the CO groups of the adjacent chain. These linkages and the general structure of the chitin unit cell are presented in Fig. 2.

Comparable data have been obtained with chitins isolated from fungi and insects by several investigators[20–24], who generally agree with Carl-



● = C o = O ● = N
a = 4.76 Å
b (fiber axis) = 10.2
c = 18.85 Å

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Fig. 2. Structure of

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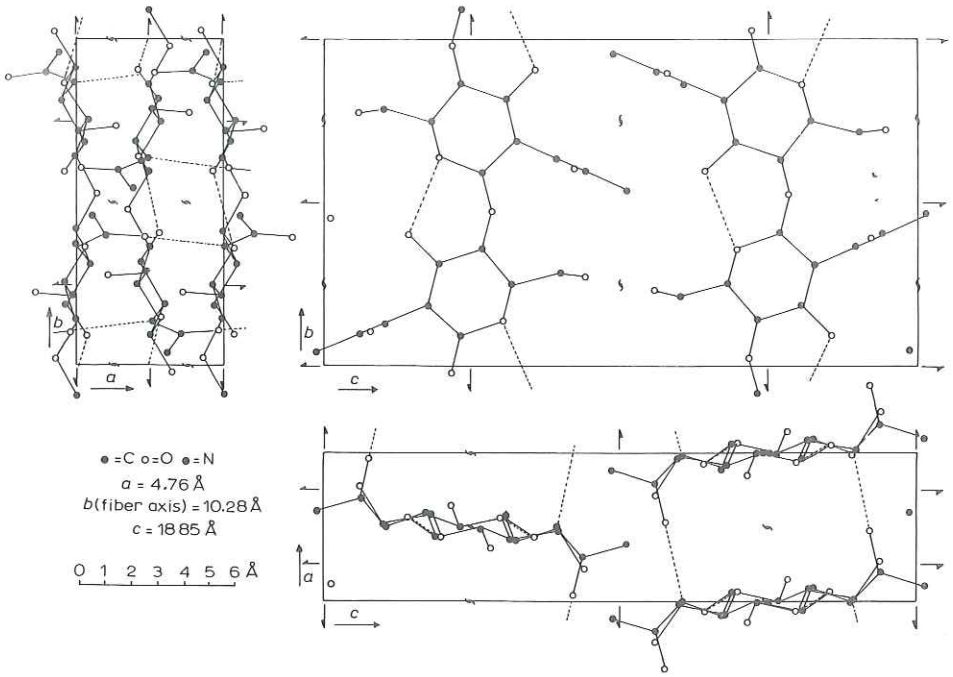

Fig. 2. Structure of the unit cell of chitin (α-chitin), after Carlström[19]. Hydrogen bonds are shown as dotted lines.

ström's interpretation. Dweltz[25] however proposed another structure, derived from X-ray-diffraction studies of the lobster tendon. The dimensions of the orthorhombic unit cell was reported as $a = 4.69$ Å, $b = 10.43$ Å, and $c = 19.13$ Å (b being the fiber axis). These dimensions are similar to those earlier reported, but the positions of the hydrogen bonds are said to be different. As shown in Fig. 3 the NH and CO groups in the neighbouring aminoacetyl chains are hydrogen bonded, as are the hydroxymethyl side-chains containing a hydroxyl group. The short hydroxyl is intra-hydrogen bonded to the oxygen of the amide group in the same asymmetric unit, in such a way that the chain structure would be straight instead of buckled[25].

This modified view of the molecular organization of the chitin chains has been carefully examined by several investigators[14,26,27], who conclude that the interpretation of Carlström seems to be the most satisfactory, especially from a stereochemical viewpoint.

X-Ray-diffraction studies of diverse supporting structures indicate three

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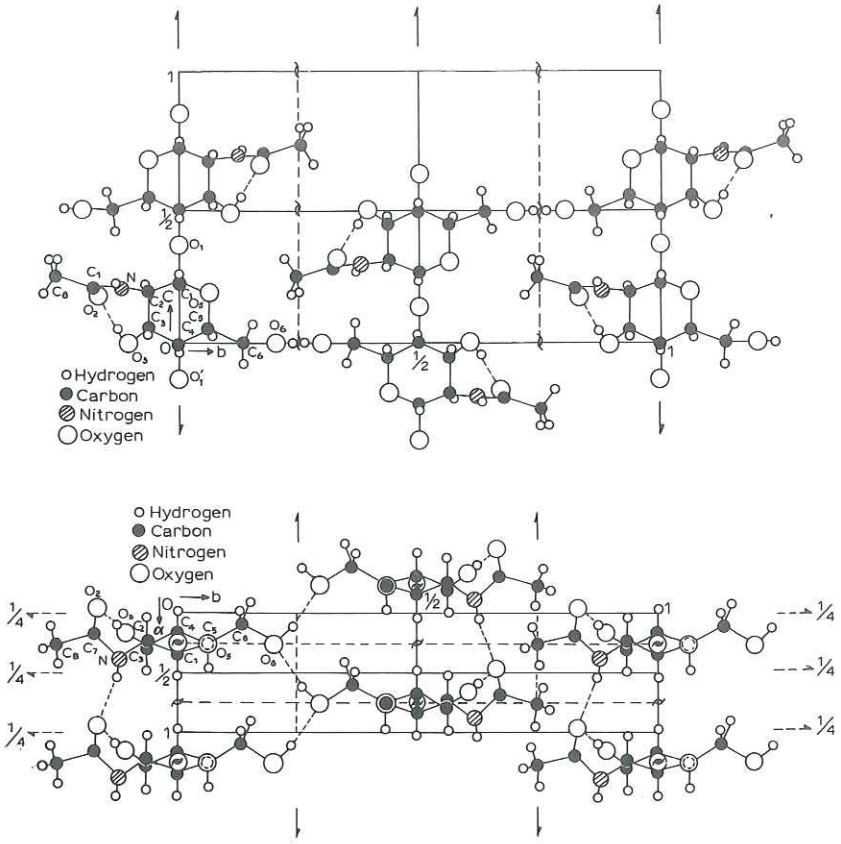

Fig. 3. Structure of α-chitin, after Dweltz[25]. Upper: the a-projection of the final structure. Lower: the c-projection of one half of the unit cell.

different types of crystallographic patterns among chitins. These patterns are presented in Fig. 4, according to Rudall[14]. The type of chitin discussed above, commonly found in arthropods and fungi, has been named α-chitin (Fig. 4B). A second type, β-chitin (Fig. 4A) has been discovered by Lotmar and Picken[23] in the chaetae of the annelid polychaete *Aphrodite aculeata*, and in the "pen" of the squid. The unit cell dimensions of isolated β-chitin are $a=9.32$ Å, $b=10.17$ Å, and $c=22.15$ Å. β-Chitin is more readily penetrated by chemical reagents and enzymes than α-chitin[12], suggesting a lower degree of "packing" of chains and a more open type of crystalline structure. Numerous free amino groups are said to exist within the chains, one or two

Fig. 4. X-Ray-diffra
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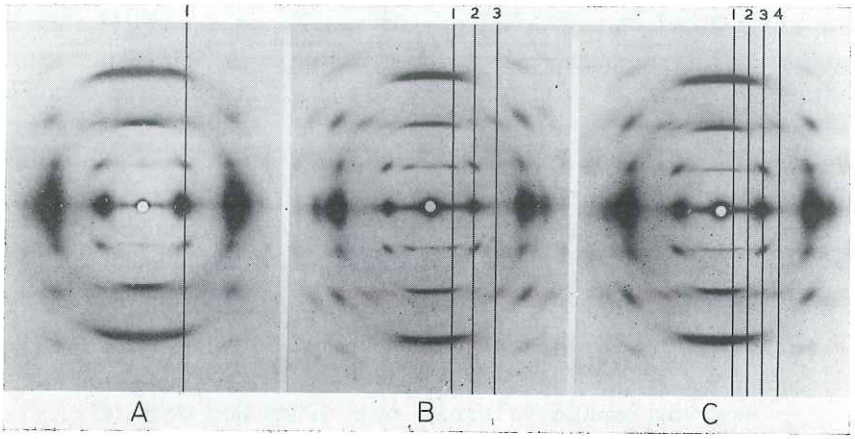

Fig. 4. X-Ray-diffraction patterns of the three main crystallographic forms of chitin, after Rudall[14]. A, β-chitin; B, α-chitin; C, γ-chitin. The essential differences between the diagrams are indicated by the approximate positions of the vertical row lines (1, 2, 3, etc.).

every five residues being unacetylated[12]. Nevertheless, after hydrolysis of β-chitin by chitinolytic enzymes, only small amounts of glucosamine occur, about 3% of the quantity of acetylglucosamine[12].

When β-chitin is dissolved in formic or nitric acid and reprecipitated by dilution, the regenerated chitin exhibits the α-chitin pattern. According to Rudall[14] and Dweltz[28], water must occupy a significant part of the crystal structure of β-chitin, perhaps one molecule per acetylglucosamine residue. A triclinic model has been proposed for the unit cell of β-chitin[28].

A third type, γ-chitin, has been recently discovered by Rudall[14,29] in the thick cuticle lining the stomach of the squid *Loligo*. Its X-ray-diffraction pattern is seen in Fig. 4C.

Rudall[14,29] proposed an attractive interpretation of these three distinct crystallographic types of chitin, differing only in the number of chitin chains in the unit cell. Fig. 5 summarizes Rudall's interpretation. In the β-chitin, each unit cell contains only one chitin chain, the different chains running in parallel directions. In α-chitin there are two chains per unit cell, running in antiparallel directions. Such an arrangement might be obtained by folding each chain of β-chitin as seen in Fig. 5E. This is supported by the β to α transformation described above, which occurs with a contraction in length of about 50%. Finally, because of the spacing of 29.2 Å in the plane of the sugar ring, given by the X-ray-diffraction diagram (Fig. 5C), the crystallites of

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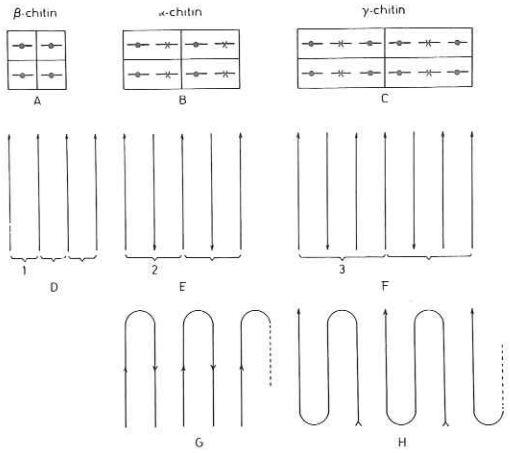

Fig. 5. Interpretation of the three main crystallographic patterns of chitin, after Rudall[14,29]. Diagrammatic representation of four unit cells of β-chitin (left), α-chitin (centre) and γ-chitin (right). In the first line, A, B and C represent basal projections of four unit cells, looking at the cell base along the length of the chains. The horizontal lines represent the plane of the sugar ring, and the signs ● and × represent two opposite directions with respect to the plane of the paper. In A (β-chitin), there is one chain per cell; in B (α-chitin), two chains per cell: in C (γ-chitin), three chains per cell. In the second line (D, E and F), chitin chains are viewed in the plane of the sugar rings and are arranged in groups of one, two or three chains. The third line represents the possible origin of the different chain orientations by folding every chain to give two (α-chitin) or three (γ-chitin) parallel segments (G and H).

γ-chitin must be formed by three chains, the central one running antiparallel between the two adjacent ones, a situation which could result from a folding of the β-chain with its extremities fixed in some way[29].

3. Distribution and chemical composition of chitinous structures

(*a*) *Detection of chitin*

Before considering the ultrastructural organization of chitinous structures, these structures should be classified. However, there is a great variety of supporting chitinous structures, especially in animals, about which little is known other than that they appear to contain chitin, usually associated with protein. The situation has been further complicated by the earlier lack of accurate analytical or histochemical methods for chitin.

The "chitosan" test is sometimes unreliable. The X-ray-diffraction method

is more highly specific, at least when appreciable amounts of chitin are present, and Rudall[24] succeeded in revealing or confirming the presence of chitin in several structures. The use of an enzymatic method, based on the highly specific and purified chitinases[30,31], has led to a systematic study of the distribution of chitin in the animal kingdom. The principle of the method earlier suggested[32,33] has been developed by Jeuniaux[30,31] so as to give accurate quantitative data. Since the method depends on the amount of acetylglucosamine liberated after complete hydrolysis, the chitin value may be lower than the actual to the extent that some non-acetylated glucosamine exists in the chitin chains.

(*b*) *Occurrence and chemical features of chitinous structures*

The available data on chitin analyses are assembled in Table I, and lead to the following comments.

(*1*) Chitinous structures are very widely distributed, particularly in animals, and exist even in less evolved taxonomic groups in which chitin was earlier believed to be absent, as in Protozoa. The biosynthesis of chitin thus appears to be controlled by early established genes, present probably in the primitive unicellular root of Metazoa[30,31]. This biosynthetic ability has been retained by a number of diblastic animals and by most of the triblastic Protostomia, but was lost at the beginning of the deuterostomian evolutionary lineage[31], with the possible exception of Tunicata, the peritrophic membrane of which is said to contain chitin[81,82]. Echinoderma, Enteropneusta, Pterobranchia, Urochordata and Vertebrata have utilized other polysaccharides such as cellulose and chondroitin sulphates, or fibrous proteins such as collagen and keratins, for their supporting structures. In plants, chitinous cell walls or structural membranes are only found in those forms, such as moulds and fungi, which like animals find considerable combined nitrogen in their food sources. In contrast, photosynthetic plants utilize nitrogen-free sugars almost exclusively for their supporting structures; chitin is however said to be a constituent of the cell membrane of some lower green plants (Chlorophycea)[40].

(*2*) Chitinous structures are mainly, if not exclusively, of ectodermal origin in pluricellular animals[24,30]; thus they form the characteristic exoskeletons of most of the "invertebrates". This is in contrast with collagenous structures which are almost entirely of mesodermal origin[24].

(*3*) Chitin rarely constitutes more than 50% of the total organic matter in chitinous structures. Higher concentrations (up to 85%) are found only in

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TABLE I

DISTRIBUTION AND CHEMICAL FEATURES OF THE DIVERSE CHITINOUS STR

Organisms	*Structures*	*Principal types of mineralizing substances*	*Chitin*	
			% organic fraction (dry weight)	*Cry type*
Fungi: Ascomyceta[b]	cell walls and structural membranes of	—	traces to 45.0[d]	
Basidiomyceta	mycelia, stalks and spores			
Phycomyceta[c]				
Imperfecti(Moniliales)				
Algae: Chlorophyceae	cell wall	—	+ ?[e]	—
Protozoa: Rhizopoda (*Pelomyxa*)	cyst wall	—	+	—
shelled Rhizopoda (Plagiopyxidae)	shell	silica	+	—
shelled Rhizopoda (*Allogromia*)	shell	iron	+	—
Ciliata	cyst wall	—	+	—
Cnidaria: Hydrozoa		—	3.2–30.3	*n*
Hydroïdea	perisarc		+	*n*
Milleporina	coenosteum	$CaCO_3$	+	*n*
Siphonophora	pneumatophore	—	+	*n*
Anthozoa (*Pocillopora*)	"skeleton"	$CaCO_3$	+	—
Scyphozoa (*Aurelia*)	podocyst	—		
			14.6[f]	—
Aschelminthes: Rotifera	egg envelope (inner membrane)	—	16.6[f]	*n*
Nematoda	egg capsule (middle membrane)	—	+	—
Acanthocephala	egg capsule	—	+	—
Priapulida	cuticle	—		
			+	—
Endoprocta	cuticle	—		
			1.6–6.4	—
Bryozoa (Ectoprocta)	ectocyst	sometimes $CaCO_3$		
			13.5	—
Phoronida	tubes	—		
			3.8	—
Brachiopoda: Articulata	stalk cuticle	—	+	*γ*
Inarticulata (*Lingula*)	stalk cuticle	—	29.0	*β*
	shell	$CaCO_3$		
			+	—
Echiurida	hooked chaetae			
			20.0–38.0	*β*
Annelida: Polychaeta	chaetae	—	0.28	—
Polychaeta (Eunicidae)	jaws	unidentified	+	*β*
Oligochaeta	chaetae; gizzard cuticle	—	+	—
all	peritrophic membrane	—		

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TABLE I

AL FEATURES OF THE DIVERSE CHITINOUS STRUCTURES IN LIVING ORGANISMS

Principal types of mineralizing substances	*Chitin*		*Main other organic constituents (figures as % organic fraction)*	*References*
	% organic fraction (dry weight)	*Crystalline type*[a]		
—	traces to 45.0[d]		polysaccharides such as glucans or mannans	33–38
—	+ ?[e]	—	cellulose	40
—	+	—	—	41
silica	+	—	—	30
iron	+	—	unidentified proteins and lipids	30, 43
—	+	—	unidentified proteins	original data
—	3.2–30.3	*n* (α?)	unidentified proteins, sometimes tanned	24, 30
	+	*n* (α)?	—	24, 44
$CaCO_3$	+	*n* (α ?)	—	24, 45
—	+	*n*	unidentified proteins	44, 46
$CaCO_3$	+	—	unidentified proteins	47, 48
—				
	14.6[f]	—	unidentified proteins	49
—	16.6[f]	*n*	unidentified proteins	30, 50, 51
—	+	—	—	52
—	+	—	tanned proteins	53
—				
	+	—	tanned proteins	54
—				
sometimes $CaCO_3$	1.6–6.4	—	unidentified proteins	30, 55, 56
	13.5	—	unidentified proteins	30
—				
	3.8	—	—	30
—	+	γ	collagen	24, 29
—	29.0	β	—	24, 30
$CaCO_3$				
	+	—	—	30
—	20.0–38.0	β	quinone-tanned proteins	23, 30, 57
unidentified	0.28	—	unidentified proteins	58
—	+	β	—	24
—	+	—	proteins	59

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TABLE I

Organisms	*Structures*	*Principal types of mineralizing substances*	*Chitin*	
			% organic fraction (dry weight)	*Cryst type*[a]
Mollusca: Polyplacophora	shell plates; mantle bristles	$CaCO_3$	12.0	—
	radula	iron	+	—
Gastropoda	shell (mother of pearl)	$CaCO_3$	3.0–7.0	—
	radula	iron and silica	19.7	α
	jaws	—	+	—
	"stomacal plates" (Opisthobranchia)	—	36.8	—
Cephalopoda	calcified shell	$CaCO_3$	3.5–26.0	β
	"pen" (*Loligo*, *Octopus*)	—	17.9	β
	jaws and radula	—	19.5	α
	stomach cuticle	—	—	γ
Lamellibranchia	shells: periostracum	sometimes $CaCO_3$	0–7.3	—
	shells: prisms	$CaCO_3$	traces–0.2	—
	shells: mother of pearl	$CaCO_3$	0.1–1.2	—
	shells: calcitostracum	$CaCO_3$	0.2–8.3	—
	gastric shield	—	17.3	—
Onychophora	cuticle	—	+	
Arthropoda: Crustacea	calcified cuticle	$CaCO_3$	58.0–85.0	α
Diplopoda	intersegmental membranes	—	48.0–80.0	α
Insecta, Arachnida	hardened cuticle	—	20.0–60.0	α
Chilopoda	unhardened cuticle	—	20.0–60.0	α
all	peritrophic membrane	—	3.8–22.0	—
Chaetognatha	grasping spines	—	+	—
Pogonophora	tubes	—	33.0	β
Tunicata	peritrophic membrane	—	+[g]	—

[a] The signs α, β and γ refer to the three crystallographic types defined by Rudall[14]; *n* means that the X-ray-diffraction pattern of chitin has been recorded, but not accurately identified as one of the former types.

[b] With the probable exception of *Saccharomyces* and related yeasts[32,33,39].

[c] With the possible exception of some Oomycetes and Monoblepharidiales[35,36].

(*continued*)

[d] Subject to considerab

[e] Chitosan test, doubtf

[f] Per cent of the total

[g] Identified by chitosa

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TABLE I (*continued*)

Principal types of mineralizing substances	*Chitin*		*Main other organic constituents (figures as % organic fraction)*	*References*
	% organic fraction (dry weight)	*Crystalline type*[a]		
$CaCO_3$	12.0	—	unidentified proteins	30
iron	+	—	unidentified proteins	60
$CaCO_3$	3.0–7.0	—	conchiolin	30, 61
iron and silica	19.7	α	tanned proteins	24, 30, 62, 63
—	+	—	tanned proteins	24, 64
—	36.8	—	unidentified proteins	65, 66
$CaCO_3$	3.5–26.0	β	conchiolin	24, 30, 67
—	17.9	β	"conchagen"	23, 30, 68
—	19.5	α	tanned proteins	24, 30
—	—	γ	—	29
sometimes $CaCO_3$	0–7.3	—	unidentified proteins	30, 61
$CaCO_3$	traces–0.2	—	conchiolin	30, 61
$CaCO_3$	0.1–1.2	—	conchiolin	30, 61
$CaCO_3$	0.2–8.3	—	conchiolin	30, 61
—	17.3	—	—	69 and original data
—	+		unidentified proteins	23, 24
$CaCO_3$	58.0–85.0	α	arthropodins+sclerotins (10–32%)	24, 32, 70–72
—	48.0–80.0	α	arthropodins (23–51 %)	24, 32, 70–72
—	20.0–60.0	α	arthropodins+sclerotins (40–76%)	22, 24, 32, 70, 71, 73–75
—	20.0–60.0	α	arthropodins+(in some parts) resilin	22, 24, 32, 70, 71, 73–75
—	3.8–22.0	—	unidentified proteins (21–47%) + mucins	76, 77
—	+	—	—	55
—	33.0	β	unidentified proteins (47 %)	78–80
—	+[g]	—	—	81, 82

[4]; *n* means that the
l as one of the former

[d] Subject to considerable variation with age and culture conditions[37,42].
[e] Chitosan test, doubtful[73].
[f] Per cent of the total dry weight of the eggs.
[g] Identified by chitosan test only.

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Arthropoda, which have exploited the aptitude of synthesizing chitin to a maximum. On the other hand, some calcified chitinous structures such as the ectocyst of Bryozoa and the shells of Gastropoda and Lamellibranchia, contain only small amounts of chitin. There are however no apparent relationships between the proportions of chitin and the degree of calcification, hardness, or flexibility of the structures.

(*4*) Chitin is associated with other polysaccharides in the cell walls of fungi. In animal forms chitin is associated with proteins, the latter frequently unidentified. In many instances of hard structures, the proteins are tanned by phenolic derivatives, namely the quinones. Collagen is rarely found in chitinous structures; the frequent independence between collagen secretion and chitin synthesis has been emphasized by Rudall[24].

(*5*) The distribution of the three different crystallographic forms of chitin does not seem to be related to taxonomy. Moreover, these three types may occur in different organs of the same animal, as in *Loligo* and *Lingula*[24,29]; it is possible that the three forms are associated with different functions[29]. According to Rudall[24], β-chitin (and probably γ-chitin) appears to be associated with collagen-type cuticles, or with collagen-secreting neighbouring tissues, while α-chitin structure completely replaces collagen-type cuticles.

(*6*) The various chitinous supporting structures exhibit striking differences in morphology, chemical composition and physical properties. The better defined chitinous structures can be classified as follows.

(*i*) *Flexible chitinous structures*

A typical flexible chitinous structure is the procuticle of the intersegmental membranes of arthropods. The cuticular sclerites also have the same characteristics just after moulting, *i.e.* before hardening by calcification or sclerotization. The chitinous procuticle is laid down by the epidermal cell layer, and it shows internal laminations visible with the light microscope. It is perforated by numerous minute ducts, the dermal ducts and the pore canals[84-88], through which the secretions of the epidermis and of the tegumental and dermal glands can reach the outside and form an outer non-chitinous layer, the epicuticle. In insects, the wax layer of the epicuticle is responsible for the water-proofing properties of the cuticle (for a detailed description of the epicuticle, see Locke[89]). The bulk of the procuticle is a chitin–protein complex, the protein fraction consisting of "arthropodins", which are not cross-linked by tanning reactions.

The innermost thin layer of the calcified cuticle of crustaceans ("membranous layer") is non-calcified; it is often referred to as endocuticle because it lies between the calcified procuticle and the epidermis, and possesses the same characteristics as the intersegmental membranes.

The peritrophic membrane of arthropods, and some other invertebrate groups, which wrap the aliments in the mesenteron and the feces in the postintestine, is an extremely thin, pliable, and hygroscopic membrane, the ultrastructure of which is described below.

A particular type of flexible chitinous structure has been recently identified in the wing hinges and elastic thoracic ligaments of the insect flight system [74,75]. Such cuticles are characterized by the presence of a rubber-like protein named resilin[90].

(*ii*) *Sclerotized chitinous structures*

The sclerites of insects and arachnids differ from intersegmental membranes by the fact that the outer layers of the procuticle are hardened by the quinone tanning of proteins. The resulting tanned proteins are the sclerotins. The outer sclerotized layers of the procuticle, adjacent to the non-chitinous epicuticle, constitute the exocuticle, while the innermost non-sclerotized layers constitute the endocuticle. There is no significant difference in the total amount of proteins between exocuticle and endocuticle of sclerites and procuticle of intersegmental membranes, but tanned scleroproteins are only found in the former layer.

The hardening of the exocuticle is often accompanied by a general darkening, *i.e.* by the formation and deposition of melanins. It has been demonstrated however that the processes of hardening and of darkening can occur separately[91,92]. It seems that melanization and sclerotization are catalysed by two different phenol oxidases[91,93].

In the hardest insect sclerites, such as in Coleoptera and Hymenoptera, the exocuticle is very thick, occupying almost the whole thickness of the former procuticle. In contrast, the sclerotized exocuticle forms only a very thin layer in the integument of insect larvae, mainly built by the endocuticle.

Histochemical observations reveal the existence, in some species, of a layer intermediate between endocuticle and exocuticle, named mesocuticle, which possesses peculiar staining properties[94]. The mesocuticle is particularly apparent and thick in arachnids[95].

In other hard chitinous structures, such as the cuticle (periderm) of Hydrozoan polyps and of Endoprocta[54], the cuticle of Priapulida[53], the chaetae

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of Annelida[57], and the jaws and radula of gastropods[62], there is also evidence for the existence of quinone-tanned proteins.

(iii) Calcified chitinous structures

One can consider the existence of two different types of calcified chitinous structures, in particular with respect to the proportions of chitin: (*1*) the sclerites of crustaceans and diplopods, in which chitin amounts to more than 50% of the organic matter, and (*2*) the shells of molluscs (except cephalopods), in which chitin generally amounts to less than 10% of the organic matter, *i.e.* only less than 0.4% of the total weight of the shell.

In molluscan shells, chitin is often associated with a fibrous protein, conchiolin, in which sclerotization by quinone tanning seems to be entirely lacking. The calcified molluscan shells are fully described elsewhere in this treatise (see Vol. 26A, Chapter IV). The chemical composition of the shells is more variable in the cephalopods. In the external calcified shell of the Nautiloidea, the proportion of chitin is low, as in other molluscan shells. In the Decapoda, the shell is an internal skeletal structure, calcified in the Sepioidea, but non-calcified in the Theuthoidea ("pen" of the squids). This internal structure is considerably reduced in the Octopoda. In these non-calcified shells, the proportion of chitin is significantly higher (about 20% of the total dry weight). According to Stegemann[68], the protein fraction, named "conchagen", of these internal structures can be converted to soluble proteins ("gelatins") by steam (143°); the remaining chitin is linked to a small protein moiety (2% of the total residue).

The sclerites of crustaceans (at least Decapoda) can be described as intersegmental membranes whose procuticle is almost entirely calcified by calcium carbonate, and to a much lesser extent by calcium phosphates. Calcium carbonate occurs as micro or macro crystals of calcite or, in rare cases, of vaterite[96]. Calcification in the cuticles of Crustacea and Mollusca has been fully studied by Travis[97–100] and is discussed elsewhere in this treatise (see Vol. 26A, Chapter IV). The hardening of the calcified cuticles seems however to be initiated by protein sclerotization, prior to the deposition of calcium salts[101]; calcified crustacean cuticles indeed always contain definite amounts of sclerotins. As a result of the calcification, the amount of proteins in the calcified cuticle is significantly lower than in the flexible procuticle or in the sclerotized exocuticle of insects. It can be considered that mineral deposits replace a large proportion of the protein material in the hardening process[32].

(iv) Non-calcified mineralized chitinous structures

There are a limited number of cases in which hardness is provided by mineral deposits other than calcium salts. The shells of some shelled Rhizopoda (Thecamoebia) and the teeth of the radula of some mollusks are hardened by iron (Fe_2O_3) or silica (SiO_2) deposits. Despite some analytical and histochemical studies[60,62,63,102,103], our knowledge of the chemistry of these structures is too limited to allow further description.

In spite of the fact that the various types of chitinous structures exhibit striking physical and chemical differences, most of them show an association of chitin with protein. The problem of chitin–protein binding and the resulting ultrastructure are therefore more fully discussed below, as well as the biochemical processes of synthesis and degradation of these structures.

4. The chitin–protein complexes

Among the chitin–protein complexes, the cuticular proteins of arthropods have received particular attention. There are at least two different components, or mixtures of components having the same properties, (*1*) a water-soluble fraction, arthropodin[22], and (*2*) a water-insoluble fraction, sclerotin[104–105]. Arthropodins are soluble in hot water, but not in cold 10% trichloracetic acid. A suitable solvent for the extraction of arthropodins is that proposed by Trim[106,107]. Amino acid analyses have been performed by several authors on arthropodins of insects[106–110] and of crustaceans[111].

The arthropodins are characterized by the absence of the sulfur amino acids, the low proportion of glycine, and the high proportion of tyrosine. They thus seem to be essentially different from all other types of structural proteins[106,107]. A number of protein fractions with different solubility and electrophoretic properties have been isolated from the larval cuticle of the insect *Agrianome*, but their amino acid composition differs only slightly[109]. Despite their electrophoretic heterogeneity, the different arthropodin fractions of the larval cuticle of *Sarcophaga crassipalpis* behave in the ultracentrifuge as a monodisperse constituent, with a molecular weight of about 7000–8000[112].

The X-ray-diffraction pattern of arthropodins show the β-configuration, which is unusual among structural proteins. Fraenkel and Rudall[22] and Richards[32,113] pointed out that the molecular spacings of this protein in the extended configuration agree with those of chitin; the identity of these lattice spacings would presumably permit a mixed crystallization, and first

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suggested the possibility of a weak bonding between chitin and protein.

Experimental evidence of covalent bonds between chitin and protein in arthropods cuticles and in cephalopod shells has been obtained during the last decade. Acetylglucosamine as well as chitin can react with α-amino acids, especially tyrosine, peptides and cuticular proteins[114,115], to give stable complexes, dissociable, however, by changing pH values. The organic material of insect cuticles, decalcified crab cuticle and squid skeletal pen, dispersed in lithium thiocyanate, can be reprecipitated by acetone to give a series of chitin–protein fractions, presumably in the form of a glycoprotein complex[3], [109,116]. The proportion of protein in these complexes varies according to the methods of isolation and the material studied, from 7.5% in the cuticle of *Cancer pagurus* to 51% in the shell of the cuttlefish[3]. Finally, different samples of chitin, prepared by alkaline digestion of insect cuticle or decalcified crustacean cuticles, have been shown, in every case, to contain small amounts of aspartic acid and histidine[3]. In the case of the larval cuticle of the insect *Agrionome spinicollis*, there are, according to Hackman[3], two histidine and one aspartic residues per 400 residues of glucosamine.

It thus appears that chitin, whether in its α- or β-crystallographic form, is covalently linked to arthropodins or sclerotins to form more or less stable glycoprotein complexes, probably through aspartyl and histidyl residues. Owing to the relative stability of these complexes in hot alkali and their instability in hot acids, the linkage could probably be as an *N*-acylglucosamine[3], that is, between a carboxyl to the NH_2 group of glucosamine. Other covalent linkages, more labile in hot alkali, could probably occur in the chitin–protein complexes[14]. The protein components of the chitin–protein complexes of insect and crustacean cuticles show, according to Hackman[3], some differences in their amino acid composition, especially in glycine, lysine, and proline, but all of them contain significant amounts of aspartic acid and histidine. It must be noted that, in the internal shells of cephalopods, the greater part of the protein moiety ("conchagen") can be removed by hot water, whereas the remaining chitin is bound to a protein containing large amounts of aspartic acid[68].

These results however have not received confirmation by recent investigators[117], who did not succeed in the isolation of chitin–protein complexes, and did not find any predominance of aspartic acid and histidine, or any other amino acid, in the residual chitin after prolonged alkali treatments of *Calliphora* cuticles and *Loligo* pens.

According to Hackman and Goldberg[109] in the case of *Agrionome* larval

cuticle, the protein fraction covalently linked to chitin amounts to 56% of the total protein. In addition to this fraction there are 3% of the total protein which is bound to other components by electrovalent bonds or double covalent bonds, 25% linked by hydrogen bonds and 2% bound by Van der Waals' forces. The remainder of the protein is not bound at all and is readily soluble in water. In certain parts of the cuticle of some insects ("rubber-like cuticles"), covalent linkages are said to occur between chitin and another type of protein, resilin[74,75].

The long radular ribbon of the Gastropoda and Polyplacophora provided Runham[60,62,63] with a convenient material to follow the sequence of events during the organization of the chitin–protein complexes. There is some histochemical evidence for the existence of covalent bonds between non-acetylated glucosamine residues of chitin and free carboxylic groups of protein, as in α-globulin and ovalbumin[118].

Whatever the exact nature of the chemical bonds between chitin and proteins, the stability of the chitin–protein complex is greatly enhanced by the sclerotization of the protein chains. This process consists of a polymerization of the polypeptide chains of "prosclerotin" by tanning with quinones. It is presumed that the prosclerotin is nothing but arthropodin. Due to sclerotization, the external part of the procuticle of an insect sclerite is transformed to exocuticle, the properties of which insure the hardness and the rigidity of the whole sclerite.

The quinone tanning of cuticular proteins is one of the physiological functions of the phenoloxidase system, which has been fully discussed elsewhere [119,120]. In the case of chitinous structures it must be emphasized that sclerotization proceeds outside the secreting epidermal cells, a rather short time after the deposition of the cuticular material in the form of a flexible chitinous structure. In arthropods, dihydroxyphenols diffuse from the epidermis through the procuticle by way of the pore canals, up to the external epicuticle where phenolases have been previously accumulated. After oxidation the resulting quinones diffuse back into the outer layers of the procuticle. The quinone reacts with a terminal amino group of a protein to form a *N*-catechol protein, which is then oxidised to a *N*-quinonoid protein. This compound reacts with the terminal amino group of an adjacent protein, forming a disubstituted derivative. Thus, a network of tanned proteins (sclerotins) is formed, including the chitin chains linked together by hydrogen bonds and covalently linked to the proteins. The degree of sclerotization determines the degree of hardness, rigidity and stability of the structure. The considerably

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higher stability given by sclerotization to chitinous structures is well illustrated by the resistance offered by the insect exocuticle to the powerful hydrolytic enzymes of the exuvial fluid during the molting processes (see below). In some cases, sulfur linkages are said to occur in the stabilization of the cuticular chitin–protein complex[121,122].

It is thus well established that the bulk of the chitinous structure is a glycoprotein in which chitin and protein are covalently linked, the proteins being, in sclerotized and many calcified structures, polymerized by a tanning process forming the chitin–sclerotin stable complexes. Beside this stable glycoprotein, there obviously exist free proteins, that can be easily extracted by water.

The study of the extent of chitin susceptibility to purified chitinases[123,124], before and after protein degradation by alkali treatment, provided a method to estimate the proportion of chitin bound or not bound to other substances in various chitinous structures[30,31]. It has been proposed to name "free chitin" that part of the chitin which is hydrolysed by pure chitinases in the intact structure, or eventually after decalcification. The "bound chitin" is only hydrolysed by chitinases after treatment with hot alkali. In most chitinous structures, the proportion of free chitin is low with respect to that of the bound chitin, the former amounting generally to only 4–30% of the total chitin. The annelid chaetae contain chitin almost entirely in the bound form, the free chitin amounting to only 0.5–2.2% of the total chitin. Two types of chitinous structures exhibit a quite different pattern; the free chitin is much more abundant in the shells of the molluscs (32–85% of the total chitin)[30,31] and in the peritrophic membranes of insects (25–68% of the total chitin)[31,77].

5. Ultrastructure

(*a*) *Flexible chitinous structures*

At the morphological level with the light microscope, the chitinous flexible structures such as insect endocuticle often appear with a lamellar organization. Numerous lamellae run parallel to the surface of the cuticle; their thickness may vary[32] from 0.2 to 10.0 μ. The lamellar structure is the result of a cyclic deposition of cuticular material by the epidermis[125–127]; morphogenesis of chitin lamellae can indeed be experimentally altered by varying light and temperature at the time of deposition[128]. Owing to the fact that, in cuticles, microfibers are only detected with the electron microscope after chem-

Fig. 6. Electron m
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Fig. 6. Electron micrograph of lamellae and microfibres shown in a transverse section of the endocuticle of *Galleria* larva, from Locke[89]. Scale = 1 μ.

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ical alteration, Richards[129,130] considered that such microfibres do not exist in normal insect cuticle. The observations of Locke[86,89] on intact insect endocuticles reveal however that lamellae do appear as being formed by sheets of microfibres (Fig. 6); these observations are confirmed by the findings of Neville[127,128]. The sheet arrangement of the microfibres corresponds to the dense part of each lamella, but the microfibres curve out at right angles between the sheets (Fig. 7). The word "lamina" has been proposed for

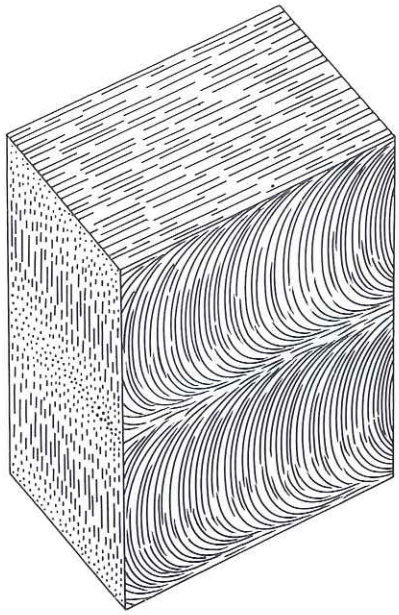

Fig. 7. Schematic representation of the arrangement of microfibres in the lamellae of a flexible chitinous structure, the endocuticle of an insect integument, from Locke[89].

that part of each lamella in which the microfibres run predominantly parallel to the surface[89]. The microfibrillar framework thus would occur in a three-dimensional pattern, an arrangement which is fundamentally different from that of plant cuticles[89]. Considering the results of autoradiographic experiments with labelled sugars and amino acids used as precursors of cuticular proteins and chitin[131], Locke[89] proposed the view that "chitin could be the molecule which is first ordered into microfibres".

A quite different interpretation has been recently proposed by Bou-

ligand[132,133] who compares the lamellar ultrastructure to a "laminated wood", in the successive superposed planes of which the direction of the microfibres rotates regularly from the bottom to the top of the cuticle; such a geometrical organization could explain any type of fibrillar disposition like those shown in Figs. 6 and 7.

When measured on electron micrographs, the dimensions of the microfibres in the flexible chitinous structures of larval cuticles would be about 25 Å in diameter[134]. This order of size seems to agree satisfactorily with the data obtained by X-ray measurements for other caterpillar cuticles[14], namely 33 Å.

The ultrastructural association of proteins with chitin has been extensively studied by X-ray methods in a series of different chitinous structures and discussed at length by Rudall[14]. It appears that there exists a number of different types of associations, revealed by different types of altered or additional X-ray reflections; all the X-ray diffraction diagrams obtained so far are however "consistent with a structure in which protein fits exactly on the pattern made by small groups of chitin chains"[14].

The cuticle of the Onychophora is, with the exception of claws and jaws, a continuous flexible and unhardened chitinous structure, surmounted by a non-chitinous epicuticle. The ultrastructure of the procuticle of *Peripatopsis moseleyi*, examined by the electron microscope, has the general characteristics of that of the arthropod procuticle and endocuticle[135].

The microfibres constituting the fundamental pattern of flexible chitinous structures are much more obvious in such structures which do not require elaborate preparation and are thus not subjected to chemical alterations. This is the case of the ecdysial membrane of moth pupae[136], of the wing cuticula of *Ephestia*[45] and the tergal cuticula of *Lepisma*[45], of the cuticle of respiratory organs of arthropods[88], and of the peritrophic membranes of arthropods[137–140]. In the latter case the peritrophic membranes are formed by systems of strands arranged in a network pattern. The size and the geometrical disposition of the strands vary with the species considered; the strands are generally 0.10–0.20 μ, and are composed of a number of microfibrils, the diameter of which[123] is about 100 Å (Figs. 8 and 9). These microfibrils are sometimes embedded in a thin amorphous film. In many cases, three systems of fibrillar strands are placed at 60° to each other, delimiting hexagonal holes (Fig. 8). In other cases, there are only two systems of strands, disposed at right angles, delimiting more or less rectangular or lozenge-shaped holes[140, 141] (Fig. 9). The arrangement of the fibrils in three sets of strands is said to be

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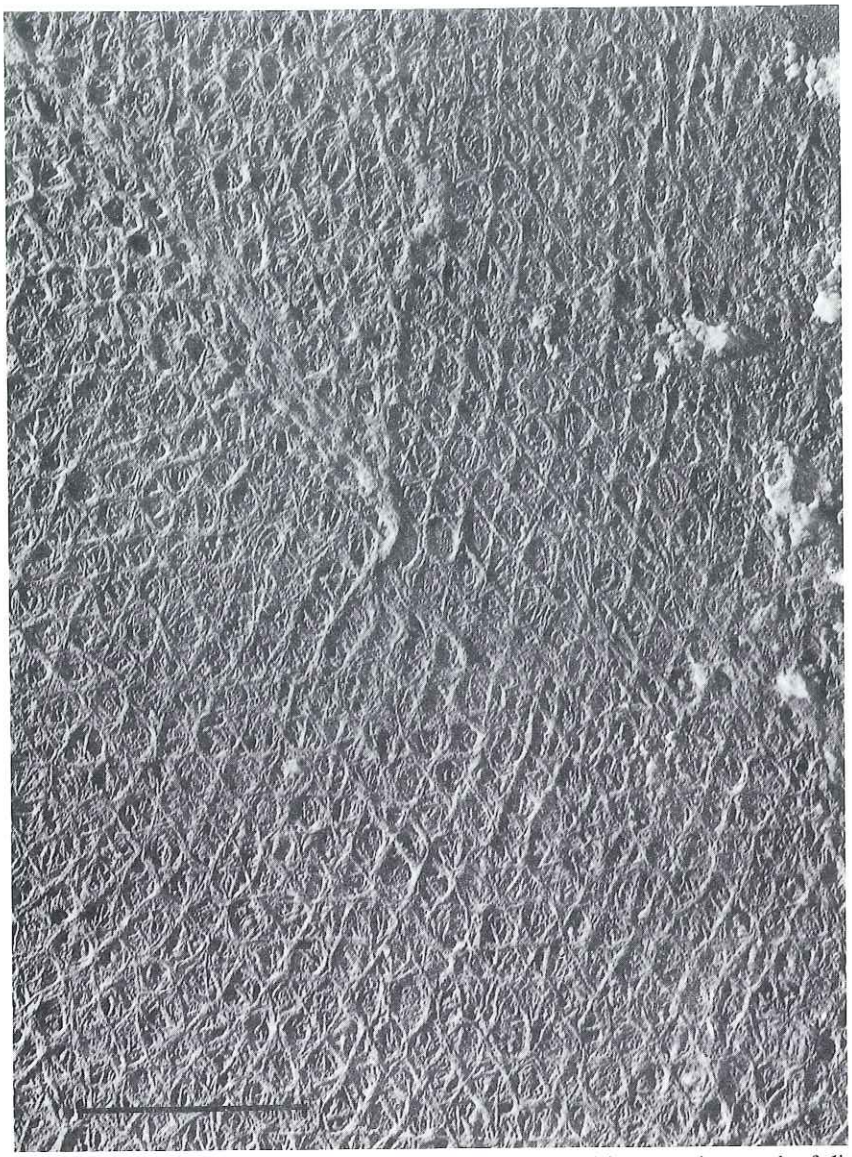

Fig. 8. Peritrophic membrane of the crab *Eriocheir sinensis* (electron micrograph of dissociated membranes deposited on to "Formvar" coated screen and shadowed with palladium; photograph Ch. Grégoire, unpublished). Scale = 1 μ.

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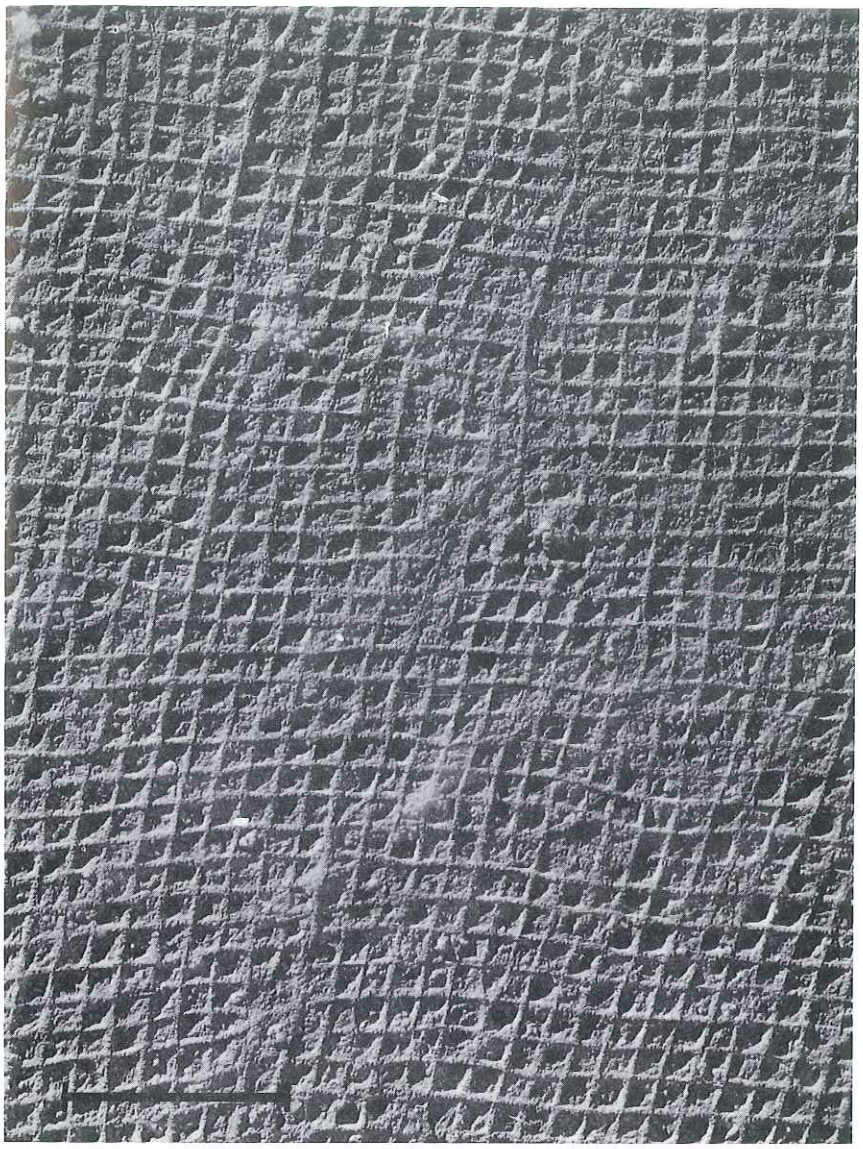

Fig. 9. Peritrophic membrane of the Diplopod *Julus albipes* (photograph Ch. Grégoire; legend and scale as for Fig. 8), after De Mets[141].

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mechanically adapted to the task of forming a tough membrane not readily torn in any direction[139], but there seems to be no correlation between type of structure and either mode of formation of the membrane or nutrition of the species[140].

As already pointed out, chitin is present in the peritrophic membranes partly in a free state and partly in a bound state, probably in the form of glycoprotein complexes. The removal of the free chitin by pure chitinases does not alter the ultrastructural pattern, while removal of proteins by alkali causes a more pronounced dissociation of the strands into separate microfibres. Successive treatments by alkali and chitinase completely destroy the structure (Jeuniaux and De Mets, unpublished). It thus seems obvious that the chitin plays the fundamental role in the structural organization of the microfibres of the peritrophic membranes.

In the flexible tube of the pogonophores, chitin is highly crystalline and exhibits the β-crystallographic configuration. In this structure, oriented long ribbon-like fibrils can also be seen with the electron microscope, after removal of the proteins by alkali and dispersion by ultrasonic vibration[78]. These fibrils are about 1000 Å wide and 200 Å thick, this unusual large size being probably explained by the high crystallinity indicated by the X-ray-diffraction pattern[78].

(*b*) *Sclerotized chitinous structures*

The ultrastructure of chitin–protein systems in the hard exocuticles of insects is under extensive examination[14,142]. Combined studies of X-ray-diffraction patterns and electron micrographs will surely be successful in the near future in elucidating the exact ultrastructural features of the chitin–protein complexes in such cuticles. The electron micrograph obtained by Rudall[142] with the ovipositor walls of Hymenoptera (see Vol. 26B, Chapter VII, Fig. 1A, p. 563) is in close agreement with the X-ray-diffraction data, the low-angle diffraction patterns originating from a hexagonally packed system of chitin rods surrounded by uniform layers of proteins. The comparative microfibrillar organization in sclerotized chitinous structures and in keratins is considered by Rudall in the present treatise (Vol. 26B, Chapter VIII). The ultrastructure of the chaetae of Polychaeta, a very peculiar type of sclerotized chitinous structure, has been studied by Bouligand[143].

(*c*) *Calcified chitinous structures*

The chitin–protein complexes found in the molluscan shells probably also

form microfibrils, dispersed in the well-known typical conchiolin structure extensively studied by Grégoire[144-147]. This conclusion can be drawn from the results obtained by Goffinet and Jeuniaux[148,149], in the case of the mother-of-pearl of *Nautilus* shell. After decalcification, the removal of the free chitin by purified chitinases does not modify the lace-like ultrastructure of the conchiolin (Fig. 10), while extraction of the insoluble nacrine (previously named nacrosclerotin[144]) of the conchiolin by mild alkaline treatments leaves an insoluble fibrous material, the "nacroine"[144]. The nacroine has

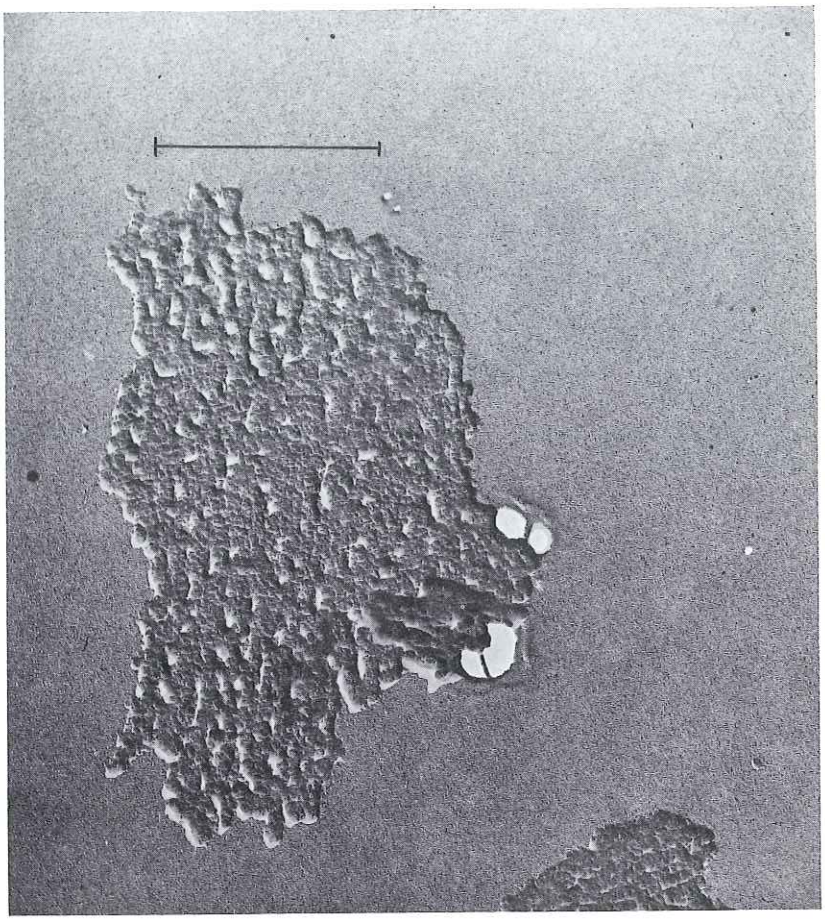

Fig. 10. Ultrastructure of conchiolin membrane of the mother-of-pearl of the *Nautilus* shell, after decalcification and treatment by pure chitinases. After Goffinet[149]. Scale = 1 μ.

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been identified as a glycoprotein formed by the association of chitin and a polypeptide mainly built up of glycine and alanine[148]. This chitin–protein complex is shown with the electron microscope in the form of discrete microfibres apparently not arranged in a continous network[149] (Fig. 11). Microfibres of the same order of size had previously been observed by Grégoire [146,147] in the mother-of-pearl of lamellibranch shells, in the residue of

Fig. 11. Microfibres of nacroïne (mother-of-pearl of *Nautilus* shell), after decalcification and treatment by NaOH 0.5 *N* at 100° during 13 h. After Goffinet[149]. Scale = 1 μ.

decalcification of calcitic prisms of lamellibranchs, and in the porcelain layer of the shell walls of *Nautilus* (see also Florkin[150]). Following the detection of chitin in all these types of shell layers[30,61], it can be presumed that these fibrils could correspond to chitin–protein complexes.

The ultrastructure of calcified chitinous cuticles is of course mainly related to the problems of calcification.

(*d*) *Concluding comments*

As a general conclusion, it seems that the ultrastructural pattern of the different types of chitinous structures is dominated by the association of chitin chains with proteins, the chitin chains being grouped in sets of one, two or three, as described earlier. Despite the opinion of some authors claiming that microfibres are the result of chemical alteration, it appears that such microfibres are obvious in many cases. A microfibrillar organization is probably a general feature of all types of chitinous structures. As far as we know, it is presumable that chitin is the prime mover of this ultrastructural fibrillar pattern.

6. Synthesis and degradation of chitinous structures

(*a*) *Formation of chitinous structures*

There are very few data concerning the mechanisms of synthesis of chitinous structures, and a comprehensive description of this process is not at the present time within our reach. Chitinous structures are probably exclusively elaborated by cells of ectodermal origin[24,30]. In many cases, the activity of the secreting cells seems to be continuous throughout life, or at least until the building of the permanent structure is completed. This is for instance the case of the periderm of Hydrozoa, the ectocyst of Bryozoa, the jaws of molluscs and the chaetae of annelids.

The epidermis of arthropods is particular in that, owing to the existence of a series of moults during the life cycle, the secretion of the cuticle is a cyclic process controlled by the moulting hormone ecdysone. The coordination of events in the formation of the insect cuticle has been clearly described by Locke[89,151] from the point of view of a developmental biologist. In the present account, suffice it to say that, a short time before moulting, epidermal cells undergo a series of modifications. Accumulation of RNA is followed by

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a decrease at the time of moulting, numerous mitoses, followed by destruction of some of the cells, and puffing of the chromosomes. The epidermis retracts and secretes at first the moulting fluid (sometimes in the form of a gel[152]) which contains hydrolytic enzymes; the epidermis secretes also a thin membrane, the ecdysial membrane. The cuticulin layers of the epicuticle are laid down soon after, and the resorption of the hydrolytic products of the endocuticle takes place, a process of absorption which is obviously important but as yet poorly understood. Successive cuticular layers of procuticle, made up of chitin–protein complexes, are laid down beneath the cuticulin layers, partly before and partly after the shedding of the old cuticle (ecdysis). The new cuticle is expanded by absorption of water, as in crustaceans[153] and aquatic insect larvae, or by air swallowing and muscular efforts as in terrestrial insects [154–156]. When the whole procuticle is secreted and fully expanded, sclerotization takes place, together or not with calcification, in the future sclerite areas (for an extensive account of this process, see Cottrell[156]). The completed cuticle remains unchanged during the intermoult period till the next moult.

(*b*) *Chitin biosynthesis*

The biochemical process of chitin biosynthesis was first elucidated by Glaser and Brown[157] in the fungus *Neurospora crassa*. Acetylglucosamine units are transferred from uridine-diphosphate–*N*-acetylglucosamine (UDPAG) as donor on the end of a preformed chitodextrin chain used as a primer. This transfer is catalysed by a chitin–UDP acetylglucosaminyltransferase (EC 2.4.1.16). The same enzymatic system has been identified in different chitin-secreting organisms or tissues such as in cell-free homogenates of larvae, prepupae and pupae of the insect *Persectania eridania*[158], in homogenates of 3-days-old larvae of the crustacean *Artemia salina*[159] and in the epidermis of the crab *Callinectes sapidus* at the time of moulting[159]. The enzymatic system leading from glucose to UDPAG has been identified in the epidermis of the migratory locust[160]. In *Persectania*, the activity of the chitin–UDP acetylglucosaminyltransferase is the highest during the late final larval instar and the prepupal instar, *i.e.* exactly during the periods of maximal cuticle elaboration[161].

It thus appears highly probable that chitin biosynthesis is catalysed by the same enzymatic system in every type of chitinous structure, and that the genetic control of the synthesis of the enzyme chitin–UDP acetylglucosaminyltransferase is the prime mover of the fitness of an organism to build up chitinous structures.

(*c*) *Biological degradation of cuticular components during the life cycle of arthropods*

It has been emphasized[89] that only a small fraction of the arthropod chitinous exoskeleton is outside the body metabolic pool, in contrast to the exoskeletal and cuticular formations of other animals. When starved for a long time, some insects such as bugs and caterpillars are able to consume part of their cuticular components[89,162]. Degradation of the cuticular material occurs normally at every moulting period. The "cast skin" (exuvium) in only composed of the epicuticle and of the hardened exocuticle in the case of insects, while the ecdysial shell of crabs and lobsters only contains half of the chitin and one tenth of the arthropodins of the intermoult shell[30,163]. Moulting indeed involves the freedom of the epidermis from the old cuticle and the catabolism of a great part of the organic material constituting the cuticle; the two processes are realized due to the elaboration and the secretion of enzymes by the epidermis, namely proteolytic enzymes, chitinases and chitobiases, in the case of larval, pupal and imaginal insect moults[152,164,165], as well as in crustacean moults [166–169].

In *Platysamia cecropia*, the epidermis secretes a gel, enzymatically inactive for a time, which becomes active when it changes to a fluid state at the end of the pupal diapause[152]. However, in *Bombyx mori*, a moth without pupal diapause, the exuvial fluid is secreted immediately in its active form[30].

In *Bombyx mori*, as probably in other insects, the biosynthesis of chitinase by the epidermis is a cyclic process. The epidermal cells are devoid of chitinase during the intermoult period; the chitinolytic activity can only be detected a few hours before the secretion of the moulting fluid[30,170]. Chitobiases on the contrary are synthetized by the epidermal cells during the whole life cycle[30].

The processes are somewhat different in crustaceans, in which chitinase and chitobiase are elaborated throughout the intermoult period, but only secreted at the apical pole of the epidermal cell at the beginning of the stage D_1 (according to the terminology of Drach[153]). During the stage D_1, the non-calcified membranous layer undergoes hydrolysis of chitin and proteins, but there remains a very stable glycoprotein complex, containing chitin linked to proteins, which imbibes water and gels[168]; the enzymes of epidermal origin accumulate in this gelled layer, from which they diffuse outward[30] during the stages D_2 and D_3. These enzymes hydrolyse most of the chitin and the proteins of the calcified cuticle, with the exception of the chitin-tanned protein complexes[30].

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In insects as in crustaceans, the degradation of the organic constituents of the cuticle is thus realized by the coupled action of two types of hydrolases: chitinases and proteolytic enzymes, which must act together in order to hydrolyse the chitin and protein molecules linked in the form of glycoprotein complexes[30].

(*d*) *Degradation of chitinous structures in digestive processes*

As emphasized above, the digestion of organic materials of chitinous structures requires the coupled action of chitinolytic and proteolytic enzymes. The adaptation of animals to a diet consisting of preys covered by chitinous cuticles or cell walls (fungi, zooplankton, arthropods), involves indeed the secretion of both types of enzymes by the glandular tissues of the digestive tract. Generally speaking, lack of chitin digestion is observed principally in animals which have adopted a chitin-free diet, such as herbivores. The distribution of chitinolytic enzymes and the correlation between the secretion of chitinase and the nature of the diet have been fully discussed by Jeuniaux[30].

(*e*) *Degradation of chitinous structures in soils, waters and sediments*

A wide number of bacteria, moulds and fungi are able to synthesize proteolytic and chitinolytic enzymes. These organisms, especially the streptomycetes in the soils, are responsible for the degradation of the chitinous structures of dead organisms. However, chitinous structures sometimes can be preserved during their burial in sediments and during geological periods. Chitin has indeed been identified in the wing-sheats of Coleoptera found in the eocene yellow amber[171] and even in a cambrian fossil pogonophore, *Hyolithellus*, which has withstood about 500 million years of fossilization[172].

7. Morphological radiation of chitinous structures

The chitinous structures, with the possible exception of those constructed by moulds and fungi, are built up by a glycoprotein framework, in which chitin is covalently linked to proteins. At this level of organization, the chitinous structures exhibit a high tensile strength but are essentially pliable and flexible, allowing movements and limited expansion. After sclerotization of the proteins, the resulting chitin-tanned protein complex gains considerable stability and confers to the structure hardness, rigidity and resistance to enzymatic

hydrolysis. Moreover, these chitin–protein complexes can be completed by the deposition of other substances, such as waxes and lipoproteins, giving the structures the properties of impermeability. Such chitinous structures have been extensively exploited by animals in the development of a number of different morphological systems or devices, assigned to a number of different functions. The biosynthesis of chitin–protein complexes has been subjected, during the course of animal evolution, to a number of "morphological radiations"[30,131].

Chitinous structures have been primarily exploited as protective envelopes, forming the theca and cyst walls of some Rhizopoda and Ciliata, the periderm or perisarc of Hydrozoa and Endoprocta, the tubes of Phoronida and Pogonophora, the shells of inarticulate Brachiopoda and of the molluscs. Chitinous structures are also used as envelopes of eggs or of latent forms of life such as cysts. By providing an adequate support for muscle insertions, chitinous structures contribute to the formation of locomotor appendages or chaetae and of prehensile and masticating organs such as jaws and radula. In Brachiopoda, a chitinous cuticle is used to insure the fixation of the organism to the substrate. Some buyoancy organs are also built up of chitin, as in Siphonophora and Cephalopoda. The chitinous peritrophic membrane of Arthropoda and annelids plays a protective role with respect to the intestinal mucosa and a role in the formation of the faeces.

The chitin–protein framework seems to provide a convenient support for calcification and silicification; as a result of this process of mineralisation of their chitinous structures, the organisms appear to realize an economy of protein material, as do many crustaceans, or an economy of both chitin and proteins, as in the case of molluscs. On the other hand, calcified exoskeleton also provides a rigid structure insuring the stability of colonies such as in Hydrozoa and Bryozoa.

At the top of the evolutionary lineage of protostomian invertebrates, the chitin–protein complexes in their most stable tanned form allow the formation of rigid planes at the expense of a minimal weight of material. Insects have developed extended portions of their teguments (paranota) which, owing to their rigidity and to their low weight, have successfully been transformed into functional wings. The wing hinges have the particular properties of a rubber-like cuticle, due to the linkage of chitin to resilin molecules. The realization of the flight system by insects is actually the consequence of the particular features of the different types of glycoprotein complexes that chitin can realize by linkage with arthropodin, sclerotin or resilin.

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The realization of a complete chitinous cuticular envelope creates for the Arthropoda problems of growth and development, as well as a problem of epicuticle replacement after abrasion. The ability to synthesize chitinolytic and proteolytic enzymes kept at the level of the epidermis, and the hormonal control of a cyclic secretion of these enzymes are biochemical features which allowed the solution of these problems and explain the success encountered by such types of chitin-covered animals.

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