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1.1 Introduction

1.1a Fibres in development

The skeletal systems of animals and plants contain extracellular fibres. Throughout development these fibres are somehow manipulated into precise directions within the supporting tissues, so as to be best adapted to their mechanical and optical functions. How this is done constitutes the subject of this book. The problem, which is unsolved, is a facet of developmental biology which is usually neglected by authors of textbooks. This control of fibre orientation occurs in plants as well as animals: in plant cell walls (from algae to timber), in the cornea in birds and other vertebrates, in human bones, in basement lamellae, in arthropod exoskeletons, and in many other systems. It is therefore a truly interdisciplinary topic in biology, relevant to researchers in all of these fields and many more. Without accurate control of fibre architecture, many animals and plants would collapse.

Those macromolecules whose functions are skeletal, such as the polysaccharides cellulose and chitin and the protein collagen, are very long. They assemble in parallel to form microfibrils, fibrils, or fibres, depending upon the particular system involved. Their intrachain backbone bonds are covalent, whereas the lateral interactions between chains are by interchain hydrogen bonds. Such assemblies have a tendency to split in a fibrous manner, like wood, if pulled in the most vulnerable direction. This is because both the strength and stiffness of fibrous assemblies are about ten times weaker when stretched across rather than along the length of the molecular backbone. Furthermore, the continuous matrices which glue the individual fibrous components together are weaker still.

For these reasons, it is vitally important that skeletal fibres are strategically oriented, so as best to be able to cope with the mechanical stresses and strains acting upon them. In addition to forming fibres, skeletal molecules also form ‘plywood’ architecture, some of which resembles the plywood made from timber scraps and some of which is considerably more advanced. The main theme of this book concerns the morphogenesis of such plywood-like laminates in the supporting structures of both animals and plants.
Plywood assemblies are extracellular, and the fibrous molecules which form them are insoluble in water. The central problem addressed in this book may be formulated as a question: How are large insoluble molecules manipulated precisely into position outside the cells which make them? A general solution to this question is proposed. I suggest that the extracellular matrix which surrounds the fibrous molecules passes through a mobile phase during development. This idea involves two further concepts: (1) The matrix is envisaged as self-assembling, and (2) it is thought to pass through a liquid crystalline phase so as to form the appropriate plywood arrangement. The matrix bonds to the long fibrous molecules, moving them into position. The mobile matrix subsequently stiffens; whereas the matrix is thought to be the prime mover in morphogenesis, the patterns which are seen in the electron microscope derive from the fibres rather than the matrix.

One type of architecture (helicoidal, defined in Section 1.3) is like a universal plywood and is the most abundant kind of regular extracellular structure in living systems. It is found in nearly all kinds of animals and plants (Chapter 2). The principle of helicoidal structure is clearly very important in biology and is relevant to researchers in a wide variety of fields.

An attempt is made in Chapter 5 (Section 5.1) to explain helicoid formation in terms of molecular shape for the specific cases of hemicelluloses in plant cell walls, proteins in insect eggshells, and collagen in bone. This is aided by comparison with synthetic cellulose derivatives. In the future we may hope for a clearer understanding of extracellular morphogenesis in terms of the complex biochemistry of proteins and polysaccharides.

In some instances parallel layers of fibres are oriented at a specific angle with respect to some defined axis of the animal or plant, often with great accuracy (e.g. 3° in beetle cuticles). Here self-assembly cannot by itself provide an explanation of how this is done. The cells which make and secrete the fibrous molecules seem to have programmed and direct control of fibre orientation. There are several types of hypotheses for this category of directed assembly, and these are summarized in Chapter 5 (Section 5.2).

The two basic kinds of control are (1) remote control by extracellular self-assembly of a succession of fibre angle changes, giving rise to a helicoid and, (2) direct cellular control of precise fibre directions by directed assembly. It is important not to confuse these two concepts, as has sometimes occurred in the literature. Both of these are examples of primary orientation, which may be defined as orientation brought about by intermolecular forces operating between macromolecules in a mobile (liquid crystalline) medium.

There is a third kind of control defined as secondary orientation (Neville, 1967c). This is a re-orientation which brings about re-
adjustment to an existing primary orientation. It is caused by some physiologically derived force. Examples are plant cell turgor pressure, muscular forces, blood pressure, growth, or some other mechanically derived force. The overriding effects of such mechanical forces cause changes to the original fibre orientations. If this happens to a regular system like a helicoid, the expected patterns may be predicted by calculation and displayed using a computer (Chapter 5, Section 5.3). The moderating effects of secondary reorientation on strength and stiffness can also be estimated.

1.1b Development beyond the cell membrane

There are many aspects to the study of developmental biology. The subject originated in classical descriptive embryology, usually taught with emphasis on the development of Amphioxus, frog, chick, and rabbit. Then the celebrated central dogma of molecular biology was established. This explained the genetic code of DNA, its transcription to messenger RNA (subsequently shown to be reversible), and its eventual translation to make proteins on the ribosomes. Some of these proteins function as enzymes which then control the chemistry of development. Gurdon (1974) has convincingly demonstrated that any nucleus from any somatic cell of an individual frog is competent to direct development into a whole frog when placed in the right cytoplasmic environment. This highlights the problem of differentiation: How do identical nuclei with identical DNA cause the cells of an individual to develop into different types? A further problem in development concerns the way in which patterns are established. These aspects are well summarized in various existing texts on developmental biology. They mostly concern events inside cells, together with cell–cell interactions. Yet there is also a whole range of developmental phenomena taking place outside of cells which is scarcely mentioned. This book is concerned with the developmental fate of the many important molecules which have been made either directly (as in the case of proteins) by the genetic code or indirectly (e.g. polysaccharides) by enzymes and then secreted through the outer cell membrane. The cell membrane arbitrarily determines what is included in this book. Thus the protein rods of the pellicle of Euglena lie just within the cell plasma membrane and hence are not included. The cellulose cell wall in such algae as Valonia, however, is outside the cell membrane and therefore is considered relevant.

The plasma membrane bounds the cell, separating cell contents (intracellular) from external products (extracellular). It consists of lipids, with a hydrophilic group at one end of each molecule and non-polar groups at the other end. The non-polar groups associate by weak van der Waals linkages to form a bi-layered
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sandwich. These links are labile (liquid crystalline), so as to per-
mit breakage and subsequent resealing during passage of large
quantities of substances into or out of a cell by pinocytosis or
exocytosis, respectively. The non-polar interface has a very high
electrical resistance (like that of paraffin wax, which has similar
bonds), so that the membrane is not freely permeable to charged
ions. The membrane also contains proteins which may protrude
to either side of the lipid bi-layer. Some of these proteins are
synthetic enzymes responsible, for example, for polymerization
of monomers to form cellulose or chitin in appropriate types of
cells. The significance of the cell membrane in extracellular de-
velopmental biology is that it represents the vital interface be-
tween the inside of the cell (where the nucleic acids have direct
control) and the outside, where more remote control methods
operate. Hence the sub-title of this book, and of this section.

1.1c The export of proteins from a cell

Proteins which are retained for use within the cell that made
them (e.g. actin or myosin in muscle cells) are synthesized on
ribosomes which lie loose in the cytoplasm. Those destined for
export from a cell are synthesized on the ribosomes attached to
the membranes of the rough endoplasmic reticulum (RER), and
they are translocated across the membrane into its lumen (known
as the cisternal space). They are packaged in vesicles which are
transported to the Golgi apparatus (by membrane flow), eventu-
ally leaving the cell by exocytosis. It has been suggested that
transport is effected by binding to microtubule proteins, moving
along a microtubule in a conveyor-belt style, by assembly of the
microtubule at one end and disassembly at the other.

A signal, consisting of a specific sequence of mainly hydro-
phobic amino acids, is recognized by a signal receptor present in
the RER membrane. If it occupies a terminal position, this signal
sequence (15 to 30 residues long) is cleaved from the rest of the
protein during translocation across the membrane. In proteins
with an internal signal sequence, such as chicken ovalbumin,
translocation does not involve cleavage. The signal sequences of
different organisms (e.g. bacteria, mice) bear strong similarities
to each other, especially in their hydrophobic nature. This sug-
gests that there is a general receptor protein in the RER mem-
brane. This protein is not found in smooth endoplasmic reticulum,
which is associated with synthesis of lipids for export. The
presence of extensive RER in a cell indicates that it is secreting
extracellular proteins.

It is therefore the signal sequence acting like a passport which
selects those proteins to be exported, including those forming
extracellular structures. Signal sequences are clearly of crucial
importance in extracellular developmental biology. Genetic re-
combination experiments in bacteria have confirmed that the addition of a signal sequence to a non-secreted protein will lead to its export. By contrast, mutants in which an extracellular protein lacks a signal sequence retain such protein in the cell.

1.1d Previous reviews

Several works have been devoted to different aspects of fibre orientation control. General works have been cited in the influential chapter on extracellular materials by Picken (1962), in the review of helicoids by Bouligand (1972), and in a study of the asymmetrical array of fibres in otherwise bilaterally symmetrical animals (Neville, 1976). Some reviews are dedicated to fibre orientation in specific systems: chitin in insect cuticle (Neville, 1967c) and cellulose in plant cell walls (Preston, 1952, 1974, 1988; Frey-Wyssling, 1976). Other reviews concern cellulose orientation in wood tracheids (Mark, 1967; Boyd, 1985) and wood analysed as a fibrous composite (Jeronimidis, 1980). Several recent works are dedicated to the function of helically wound fibres in animals (Alexander, 1987, 1988; Wainwright, 1988). There is a chapter dealing with molecular aspects of extracellular materials in the book by Alberts et al. (1989). Molecular and mechanical functions are integrated in two books which are highly recommended reading: Wainwright et al. (1976) and Vincent (1982).

1.2 Aims and significance

The academic purpose of this book is to present a novel account of fibrous composites (materials which resemble fibreglass) in animals and plants. It aims to attract the interest of a wide range of workers, such as developmental biologists, biochemists, plant physiologists, animal physiologists, botanists and zoologists, liquid crystal chemists, biophysicists, and materials scientists. The objective is to promote new thinking and inspiration wherever fibrous structure is relevant to these fields, by enticing research workers to read about similar work on materials different from the ones they normally use.

The specific aims of the chapters are as follows. The first chapter includes definitions of the principles of fibres and fibrous composites, together with the architectures which they produce. Chapter 2 covers the distribution of various prominent types of fibrous architecture; this reveals the importance, in particular, of helicoidal plywood. The accent in this book is on development rather than function; however, chemical, optical, and mechanical properties are discussed in Chapter 3. The fourth chapter concerns the probable involvement of liquid crystals in the formation of fibrous composites. In Chapter 5 we begin to see an integration of knowledge – chemical structure and shape, liquid crystalline self-assembly, and formation of fibrous composites.
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The problem may be stated in question form: How does chemistry create architecture outside cells? This opens new avenues of thought, such as how the shapes of hemicelluloses in plant cell walls and of proteins in insect eggshells relate to helicoidal structure. It is exciting that the work with insect eggs provides a link to molecular genetics. In Chapter 6 I search for generalizations. Architecture, for instance, may override chemistry; two cathedrals can be architecturally the same and yet be built of different rocks, and two different cathedrals may be built from the same rock. An attempt is made to relate overall molecular shapes to fibrillar architecture and to interlink the main types of structures in animals and plants via types of liquid crystals.

In addition to academic aims there are also applied motives for studying fibres, some of which lead to funding of research. Detailed knowledge of plant fibrous systems helps in understanding the mechanical properties of commercially useful cell walls, such as flax, jute, hemp, and cotton in the rope and textile trades. The paper and gum industries are also based on plant fibrous products. Fibrous studies are involved in gaining a better understanding of the theoretical strength of timber, the method of breakdown of wood by fungi, and the functions of fibre in human diet.

There are also applied reasons for studying some of the animal fibrous systems (e.g., the silk industry). Important vertebrate fibrous systems include human bone, tendon, cornea, teeth, artery walls, and extracellular matrix. Several diseases are due to malfunction of the basement membrane (see Section 2.2f). Some insecticides work by altering the fibrous composite matrix in insect cuticle, while some weedkillers have similar effects on plant cell walls. For the future there is the prospect of trying to copy fibrous composites in vitro by biomimicry. And for geologists, whose interests extend to the past, extracellular fibrous structures are important because often they are the only parts which fossilize.

1.3 Definitions and diagrams

1.3a Layer deposition sequences

Many extracellular fibrous composites are secreted layer by layer in a sequence resulting in a laminate. Figure 1.1 shows some examples, with time’s arrow indicated; this sometimes points in an unexpected direction, and mistakes occasionally occur in the literature. Diagrams are given in Figure 1.1 for plant cell walls, insect cuticle, vertebrate cornea, moth eggshell, and fish eggshell.

Invertebrates mostly secrete from a two-dimensional epithelial cell surface, so that the natural product is a laminated structure. The products of neighbouring cells are pooled (e.g. in insect cuticle) so as to avoid weakening junctions (Fig. 1.2C). The implication of laminated structure is that it may be broken down or
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Figure 1.1. Simple diagrams of the sequence of deposition of some fibrous composites. Many extracellular supporting structures are laminates, secreted layer by layer in sequence (shown by the time arrows, which run from the older to the newer deposits). (A) Two neighbouring plant cells, surrounded by cell walls. The primary wall is secreted first (PW), and the secondary wall (SW) is secreted after the cell has finished enlarging. (B) Insect cuticle secreted by a cellular epithelium (epidermis). The exocuticle (EX) is secreted before an ecdysis and the subsequent expansion. This is followed by the endocuticle (EN), which is not expanded after secretion. (C) Cornea of the vertebrate eye. The epithelial cells on the outside of the eye secrete the primary stroma (shaded) via their inside faces. (D) A moth eggshell chorion (dashed) surrounds the oocyte (dotted), but is itself secreted by the maternal follicle cells of the female moth, which surround the shell. (E) A fish eggshell is also sheathed by the follicle cells of the female fish, but the shell layers are secreted from the inside by the oocyte; the first layer deposited is the cortex radiata externus (CRE), followed by the cortex radiata internus (CRI), which is helicoidal.

renewed only from its newest surface (e.g. during moulting in arthropods).

In vertebrates, by contrast, the secretion of fibrous composites is complicated by the invasion of cells. These cells become arranged in isolated suspension in three dimensions within the composite. Examples include fibroblasts (which secrete collagen) in the primary stroma of eye cornea, cells in sea-squirt tests (Fig. 2.29), and bone, which contains osteoclasts to break bone down, and osteoblasts to renew it. The shape of a bone can thus be continually remodelled from within. Although arthropod cuticle can change its thickness without a moulting, moulting is needed to achieve changes in external shape.

1.3b Cellular and fibrous interrelations

Figure 1.2 shows some spatial relations between skeletal materials and the cells which secrete them. Figure 1.2A shows keratinocyte cells which form vertebrate structures such as horn, skin, feather, quill, and beak. These are not in fact examples of extracellular structures, because the fibrous protein keratin is found inside the cells. Plant fibres are built of individually boxed cells (Fig. 1.2B) (e.g. jute and hemp). Despite keratinocytes being
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Figure 1.2. Simple drawings of interrelations of cells and skeletal fibres in some major living systems. (A) A group of keratinocytes typical of vertebrate tissues (skin, horns, nail, hoof, baleen, hair, claw, beak, feather, etc.). The cells are strengthened by fibres of the protein keratin, which is found inside the cells – never outside them. (B) The individually boxed cells of plants. Each cell is enclosed by the fibrous composite plant cell wall which it has itself secreted. Fibres, usually of cellulose, are embedded in a complex extracellular matrix, itself also mainly polysaccharide. (C) Sheets of cells (epithelia) combine their secretory efforts to produce laminated cuticles in insects, spiders, crabs, and other arthropods. The fibrous composite cuticle has chitin fibres set in a complicated matrix of proteins. Neighbouring cells pool their extracellular products. Cuticle is secreted exclusively from one face (the outer or apical face) of the epithelium; the cells are highly polarized. (D) In vertebrates, the cells (white) which secrete bone and cartilage are isolated by the products of their own secretions (stippled). They keep in touch via communicating cellular processes, running through the three-dimensional matrix. Unlike in arthropods, secretion is not confined to one localized part of the cell surface.

intracellular and zoological in origin, and plant cell walls being extracellular and botanical in origin, they nevertheless have a feature in common: both require long cells, with molecules lined up in parallel to form fibrous structures.

Figures 1.2C and 1.2D respectively show a pooled laminate secreted by the two-dimensional surface of an epithelium compared with a three-dimensional pooled matrix, as in bone and cartilage.

1.3c Microfibrillar crystallites as construction units

Different architectures are built with differently sized construction units (Neville, 1975b). In the case of cholesteric liquid crystals (see Section 1.3g and Chapter 4) of PBLG (polybenzyl-l-glutamate) (Fig. 4.7A), the construction unit is the single molecular chain. Biological fibrous composites are built with larger units, such as microfibrils or crystallites (e.g. cellulose in plant cell walls, or chitin in insect cuticle). These are of the order of 3 nm in diameter, in the case of chitin consisting of 19 molecular chains hydrogen-bonded in parallel. Details of the structure of a chitin chain and crystallite are given in Figure 1.3. Other composites are built of fibrils (bundles of microfibrils). Examples are crab cuticle (Fig. 2.23), beetle cuticle layers (Fig. 2.39), the cuticle in Rhipita pachyptila, which belongs to the new phylum Vestimentifera (Gaill, Herbage, & Lepescheux, 1988), tunicate test cellulose (Fig. 1.14), and collagen in frog tadpole cornea (Fig. 2.8). In the case of crab cuticle the fibrils are 25–50 nm in diameter.
1.3 Fibrous composites

A main theme of this book is fibrous composites, which function like fibreglass (Section 3.2). A good example is the rubber-like cuticle in insects, which contains crystallites of chitin embedded in a matrix of the protein resilin. This is a rubber with an almost perfect elastic efficiency (97% resilience). Because the crystallites are very small in diameter – 3 nm is much thinner than a cell membrane – how do we know that the crystallites are chitin and the matrix resilin? Figure 1.4 shows an electron micrograph of pure resilin from a locust; no crystallites are seen. Figure 1.5 shows a micrograph of a sample of rubber-like cuticle from a locust – known from chemical analysis to contain only chitin and resilin (Neville, 1963b). Hence, by comparing the two, we see that the crystallites resolved in Figure 1.5 must be chitin. More convincingly, the volume fractions of crystallites and matrix may be measured from the micrographs and multiplied by the respective dry densities of chitin and resilin. This gives good agreement with quantitative chemical analysis (Neville, 1984). The chitin remains unpenetrated by heavy-metal stains such as uranyl acetate or potassium permanganate; this indicates that the chitin is highly crystalline, and this is confirmed by the detailed X-ray diffraction diagrams obtained from tendon samples.
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Figure 1.4. (top left) Electron micrograph of a section through one of the large pieces of resilin (a protein rubber) used in storing energy for jumping in an adult oriental plague flea (Xenopsylla cheopis). There is no chitin in this sample. Compare with Figure 1.5. Stained with uranyl acetate and lead citrate. From Neville (1984), by permission of Springer-Verlag, Heidelberg.

Figure 1.5. (top right) Electron micrograph of section through one of several large pieces of resilin used in storing energy for flight in an adult desert locust (Schistocerca gregaria). Chemical analysis shows that this sample contains only two components: protein resilin and polysaccharide chitin. By comparison with the pure resilin in Figure 1.4 the chitin crystallites must be the unstained (white) areas seen in cross-section. The resilin matrix stains with uranyl acetate and lead citrate. This is a classical example of a natural fibrous composite. From Neville (1984), by permission of Springer-Verlag, Heidelberg.

Figure 1.6 (bottom) Electron micrograph of an oblique section through the endocuticle of the fifth instar larval hind tibia of a giant water bug (Hydrocorynus colombei; Belostomatidae). The architecture is pseudo-orthogonal and is visualized by chitin microfibrils as well as pore canal shapes (which, when crossing through parallel layers, appear like an aerial view of ships). Two major fibrous layers are seen; the change in direction between them is via approximately 90° of helicoid. Stained with potassium permanganate and lead citrate. Unpublished micrograph by B. M. Luke and A. C. Neville.

of insect cuticle (Rudall & Kenchington, 1973; Neville, 1975a). In order to function as a good fibrous composite, the fibrillar component must be as stiff (crystalline) as possible. For further details, see Section 3.2a and Rosen (1974).

1.3e Parallel fibre systems

Fibrous materials have very well oriented molecules which need to be straight to be able to line up in parallel (Fig. 1.6). In the case