THE CONTROL OF GROWTH & DIFFERENTIATION IN PLANTS

2nd Edition

P. F. Wareing
I. D. J. Phillips

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SECOND EDITION

by

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Preface to the Second Edition

THE STEADY increase in our knowledge and understanding of plant development which has occurred since the first edition of this book almost 10 years ago has necessitated its extensive revision. In the present edition large sections have been rewritten and extended to bring the information up to date, and an additional chapter on Phytochrome and Photomorphogenesis is now included. There has also been extensive rearrangement of the material to give a more logical and coherent presentation.

We are indebted to our colleagues Dr. M. A. Hall and Dr. P. F. Saunders, and to Professor J. Heslop-Harrison and Professor J. Zeevaart, for reading various sections of the revised manuscript and for their helpful and constructive criticisms.

Preface to the First Edition

THE PHENOMENON of development, in both plants and animals, presents some of the most challenging unsolved problems of biology and is one of the remaining areas in which it cannot yet be said that we have made a decisive "breakthrough", comparable with the recent advances in biochemistry and molecular biology. Nevertheless, during the past 30 or 40 years there has been a steady advance in our understanding of the physiology of growth and differentiation in higher plants, so that there is now a considerable body of well-established knowledge in this field. It is generally accepted that some knowledge of the physiology of plant growth and differentiation is important for all students of the botanical sciences, and sections on this subject are commonly included in textbooks of plant physiology. The developmental approach is now also given more prominence in the contemporary teachings of plant form and structure than in the past, but there have been few attempts to bring together both morphological and physiological approaches within the same volume, and this we have attempted to do. Unless we attempt to relate the two approaches to each other, morphological and anatomical accounts of growth and differentiation must remain largely descriptive in nature, whereas our aim should clearly be to understand the processes underlying and controlling the structural changes. Conversely, physiological and biochemical studies which are not related back to developmental processes in the plant are liable to lose relevance and biological significance.

While it is true that we have found it convenient to concentrate on the structural aspects of development in the first few chapters, nevertheless we have attempted, throughout the book, to base our whole approach upon the growth and differentiation of organs and tissues, and the major developmental changes in the plant as a whole. Thus, after giving a brief account of the chemistry and biochemistry of plant hormones, we return to consider their role in the control of growth and differentiation at various levels. We then consider the factors regulating the major phase-switches in the development of the plant as a whole, viz. those controlling flowering, dormancy and senescence.

Finally, in the last chapter, we examine some basic problems in plant development, including the nature of the control mechanisms in differentiation. There are large gaps in our understanding of some of the most important aspects of development, such as the nature of the processes controlling the orderly sequence of changes so characteristic of development of all organisms. The discussion on such topics is, of necessity, largely speculative, but current thought in molecular genetics and theories of gene activation and repression derived

from studies on micro-organisms enable us to formulate the problems in more precise terms than has been possible hitherto.

The book is intended primarily as an introduction to plant growth and differentiation for undergraduate students. While we have attempted, so far as possible, to give the supporting evidence for statements, references to the individual pieces of research upon which we have drawn are reduced to a minimum. We believe that an over-extensive chronicle of research workers' names and dates only serves to distract the student's attention from the main theme. We are confident that the many unnamed researchers whose work has made this book possible will not be offended. To assist the student, however, we have provided a list of additional reading, from which he can obtain detailed reference lists.

This book has only been made possible by the kind assistance of many persons in different ways and we make grateful acknowledgement of their help. We should mention specially, however, Professor A. W. Galston, Professor H. Heslop-Harrison, our editors, Professor G. F. Asprey and Dr. A. G. Lyon, and our colleagues, Dr. M. A. Hall and Dr. P. F. Saunders, all of whom have read sections of the book and have made most helpful suggestions. Responsibility for any errors which may have crept into the book must remain ours, however. We also wish to thank Miss M. Bigwood for her skilled assistance in the preparation of some of the diagrams.

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CHAPTER 1

Growth in the Higher Plant

INTRODUCTION

We are all so familiar with the remarkable changes which occur during the life cycle of a plant, from germination to fruiting and senescence, that we tend to take the phenomenon of development for granted, so that it may cease to excite our wonder. Nevertheless, the orderly succession of changes leading from the simple structure of the embryo to the highly complex organization of the mature plant presents some of the most fascinating and challenging outstanding problems of biology. In this book we shall be concerned primarily with describing and examining what is known about the processes underlying and controlling plant development.

Before we can proceed, however, it is necessary to define certain terms, which are not always used in precise senses. *Development* is applied in its broadest sense to the whole series of changes which an organism goes through during its life cycle, but it may equally be applied to individual organs, to tissues or even to cells. Development is most clearly manifest in changes in the form of an organism, as when it changes from a vegetative to a flowering condition. Similarly, we may speak of the development of a leaf, from a simple primordium to a complex, mature organ.

Plant development involves both *growth* and *differentiation*. The term growth is applied to *quantitative* changes occurring during development and it may be defined as an irreversible change in the size of a cell, organ or whole organism. The external form of an organ is primarily the result of *differential growth* along certain axes. However, during development there appear not only quantitative differences in the numbers and arrangement of cells within different organs, but also *qualitative* differences between cells, tissues and organs, to which the term *differentiation* is applied. Differentiation at the cell and tissue level is well known and is the primary object of study in plant anatomy. However, we may also speak of differentiation of the plant body into shoot and root. Similarly, the change from the vegetative to the reproductive phase may also be regarded as another example of differentiation. We shall, therefore, apply the term differentiation in a very broad sense to any situation in which meristematic cells give rise to two or more types of cell, tissue or organ which are qualitatively different from each other.

Thus, we may say that *growth and differentiation are the two major developmental processes.* Usually growth and differentiation take place concurrently during development, but under certain conditions we may obtain growth without differentiation, as in the growth of a mass of callus cells (Chapter 6).

The problems of development can be studied in a number of different ways, but basically there are two major types of approach, viz. (1) the morphological and (2) the physiological and biochemical. Developmental morphology and anatomy were formerly largely concerned with describing the visible changes occurring during development, but current interest is mainly directed to trying to understand the factors and processes determining plant form, using experimental techniques, such as surgery, tissue culture, autoradiography and so on. However, development cannot be fully understood without a study of the manifold biochemical and physiological processes underlying and determining the morphological changes, and it is these latter aspects of development which form the main subject of this work.

The experimental morphologist often uses the term *morphogenesis*, which, in the literal sense, is concerned with the origin of form in living organisms. However, by the term "form" should be understood not only the gross external morphology of the plant, but its whole organization, which may be recognized as existing at several different levels; thus, we may recognize (1) the structural organization of the individual cell, as shown by electron microscopy, (2) the organization of cells to form tissues, and (3) the organization of the plant body at the macroscopic level. Moreover, in the study of morphogenesis we are concerned not only with observable changes in form and structure but also with the underlying processes controlling the development of organs and tissues, and insofar as these processes must ultimately be explainable in terms of physics and chemistry, this aspect of morphogenesis is identical with developmental physiology and biochemistry. However, at the present time our knowledge of the molecular basis of morphogenesis is very fragmentary and we know very little about the physiological and biochemical processes regulating, for example, the initiation and development of leaves.

When we come to consider the physiology of development, we find a further dichotomy of approach. On the one hand, a considerable body of knowledge has been acquired about the role of hormones as "internal" factors controlling growth and differentiation; on the other hand, the profound importance of environmental factors, such as day length and temperature, in the regulation of some of the major phases in the plant life cycle has been clearly demonstrated, although there is considerable evidence that a number of environmental influences are mediated through effects on the levels and distribution of hormones within the plant.

It is axiomatic that the plant body at any given stage is the resultant of the interaction between the inherent (genetic) potentialities of the species and the external factors of the environment. Thus, we cannot say that certain characteristics of the plant are determined genetically, whereas others are environmentally determined, since *all* its characteristics are affected by both genetical and environmental influences. However, it is quite legitimate to say that some *differences* between plants are primarily genetically determined whereas

others are due to environmental factors. Thus, the lack of chlorophyll in a plant may be caused by a mutation affecting chlorophyll biosynthesis. On the other hand, a plant may lack chlorophyll because it has been grown in the dark, so that it is etiolated. But it must be emphasized again that the development of a normal green leaf requires both the appropriate genetical factors and certain environmental conditions, including light.

When we speak of the genetical potentialities of the species we must include not only genes located in the nucleus, but also cytoplasmic factors. Certain characters of the plant, including some chloroplast characters, show cytoplasmic inheritance. This fact should not surprise us unduly, since it is now well established that chloroplasts contain DNA and are probably self-replicating organelles. In this book we are not primarily concerned with genetical aspects of morphogenesis, but in all our discussions of this problem it is a basic assumption that, in the final analysis, development involves the expression of the information stored in the genes.

THE LOCALIZATION OF GROWTH

One of the essential characteristics of organisms is that they are able to take up relatively simple substances from their environment and use them in the synthesis of the varied and complex substances of which cells are composed. It is this increase in the amount of living material which is basically what we mean by growth. At the cellular level the increase in living material normally leads to an increase in cell size and ultimately to cell division. These two aspects of growth are seen in their simplest form in unicellular organisms such as bacteria, unicellular algae and protozoa, where growth leads to enlargement of each cell which then divides and the process is repeated.

When we come to consider the growth of multicellular organisms, such as the higher plants, the situation is much more complex. It is true that here, also, growth ultimately depends on the enlargement and division of individual cells, but not all cells of the plant body contribute to the growth of the organisms as a whole, for growth is restricted to certain embryonic regions, the *meristems*. This restriction of the growing regions is probably related to the fact that mature plant cells are normally surrounded by relatively thick and rigid cell walls, and many cells of mechanical and vascular tissues are, of course, non-living. These facts would probably render co-ordinated growth, involving both cell division and cell enlargement, difficult in an organ, such as a stem once a certain stage of differentiation had been reached. We shall see later that most living plant cells retain the capacity to divide under certain conditions, but even if they do divide the daughter cells do not necessarily increase in size, unless they are relatively thin-walled cells which are able to revert to the embryonic or "meristematic" condition. In having rather strictly localized embryonic regions higher plants differ from animals, where growth typically occurs throughout the organism as a whole.

This difference between higher plants and animals is no doubt related to the basic differences in the modes of nutrition of the two groups. Because they have to take up water and

mineral salts from the soil, the autotrophic land plants must necessarily be rooted and sessile, whereas most animals have to forage for their food, whether they are herbivorous or carnivorous, and they need, therefore, to be mobile. This requirement for mobility in animals which forage for their food, in turn, demands that they should have flexible bodies, whereas the plant body can be much more rigid and indeed it needs to be so in erect-growing plants, especially in large forest trees. This rigidity and firmness of the plant body depends upon the presence of relatively thick and firm cell walls, whether in the living cells of the leaf, for example, or in the non-living cells of mechanical tissue of the stem. (The rigidity of those tissues consisting mainly of living cells depends, of course, on the turgidity of the cells and not simply on the mechanical properties of the walls, but even in such tissues a cell wall is an essential requirement for the attainment of the turgid condition.) On the other hand, in aquatic plants, whether they are lower plants or angiosperms, nutrients may be absorbed from the surrounding water directly into the shoot, so that they may be free-floating, and the mechanical tissues are usually less well developed than in land plants.

A number of different types of meristem may be recognized in the plant body. The axial organs, the stems and roots, have *apical meristems*, i.e. growth in length is restricted to the tip regions and the new tissue is added to the plant body on the proximal side, so that the pattern of growth may be described as *accretionary*. The apical meristems of the stem and root usually remain permanently embryonic and capable of growth over long periods—for hundreds of years in some trees. Consequently we may describe these as *indeterminate* meristems.

On the other hand, other parts of the plant, particularly the leaves, flowers and fruits, show rather different patterns of growth and they are embryonic for only a limited period before the whole organ attains maturity. Thus, the growing regions of such organs are sometimes referred to as *determinate* meristems. In such organs the pattern of growth resembles that of animals in that, firstly, there is an embryonic phase of limited duration and secondly, in such organs growth is more generalized than in stems and roots.

The presence of indeterminate meristems, together with the capacity for forming branches, each with its apical meristem, gives the plant body a much less precise and definite form than is the case for the animal body. Indeed, the general form of the plant body resembles a colony of coelenterates, such as corals, rather than that of an individual higher animal. On the other hand, the organs showing determinate growth, such as leaves and flowers, generally show much more precise morphology and may have fairly precise numbers of parts, such as petals.

In addition to classifying meristems as indeterminate and determinate we may classify them in various other ways. For example, we may distinguish the apical meristems of stems and roots, from the *lateral meristems*, comprising the cambium and phellogen (cork cambium). In some plants there are *intercalary meristems*, inserted between regions of differentiated tissues. One of the best known examples of this type of meristem is seen in grasses, where the internodes and leaf sheaths continue growth in the basal region, after the upper parts have become differentiated. The structure of some of these meristems will be described in more detail in Chapter 2.

CELL DIVISION AND CELL VACUOLATION

The growth of a multicellular plant involves both increase in cell number, by cell division, and increase in cell size. These two aspects of growth have no sharp spatial boundaries; however, in the apical regions of shoots and roots cell division occurs most intensively towards the extreme tip of both organs, whereas the region of most rapid increase in cell size is in a zone a few millimetres back from the tip (Fig. 1.1). In organs of determinate growth, such as leaves and fruits, these two aspects of growth tend to be separated in time, so that there is an early phase in which cell division is predominant, followed later by a phase when cell division ceases and there is active increase in cell size. The greater part of this increase in size is due to vacuolation, i.e. by water uptake, and as a result the cytoplasm may come to be limited to a thin boundary layer against the cell wall.

In the tip regions of roots and shoots in which cell division predominates, the cells are relatively small, and have prominent, spherical nuclei lying towards the centre of the cytoplasm, which is non-vacuolated and tends to be densely staining; the cell walls are thin. The details of the process of mitosis by which the nucleus divides need not be described here. As a result of division, each of the two daughter cells is only half the size of the parent cell. These cells then proceed to enlarge, but such cell growth involves the synthesis of cytoplasm and cell wall material and not vacuolation.

Since the number of cells in the zone of cell division tends to remain fairly constant (at least over limited periods), it is clear that not all the daughter cells formed in this zone retain the capacity for unlimited further division. The situation is perhaps best illustrated by reference to plants which grow by a single apical cell, such as certain algae and the bryophytes and some pteridophytes where it can clearly be seen that division of the apical cell results in one cell on the outside which becomes the new apical cell, and a second daughter cell, on the proximal side, which gives rise to the differentiated tissue of the thallus or shoot. This latter daughter cell usually undergoes several further divisions but ultimately the derivative cells lose their capacity for division. Thus, whereas the apical cell remains permanently meristematic, the derivative cells are capable of only a limited number of further divisions. The situation must be analogous in the more complex apices of gymnosperms and angiosperms, where there is normally a number of initial cells, i.e. cells which remain meristematic and undergo repeated division, but it is more difficult to recognize which of the daughter cells is destined to remain meristematic and which will give rise to mature tissue. The problem of why cells in the initial zone remain permanently embryonic or meristematic, whereas the derivative cells on the proximal side are capable of only a limited number of further divisions is an intriguing one, but it remains unsolved at the present time.

At a certain distance from the apex, in both shoots and roots, the process of vacuolation commences and, as a result of this process, the root cells of *Allium* may increase in length from 17 μm to 30 μm and in volume by 30-fold. In other tissues the cells may increase up to 150-fold in total volume during vacuolation. It appears that this great uptake of water during cell extension is essentially governed by osmosis, and if we apply the usual concept relating to water uptake by cells, then, in general, the ability of the cell to take up water is

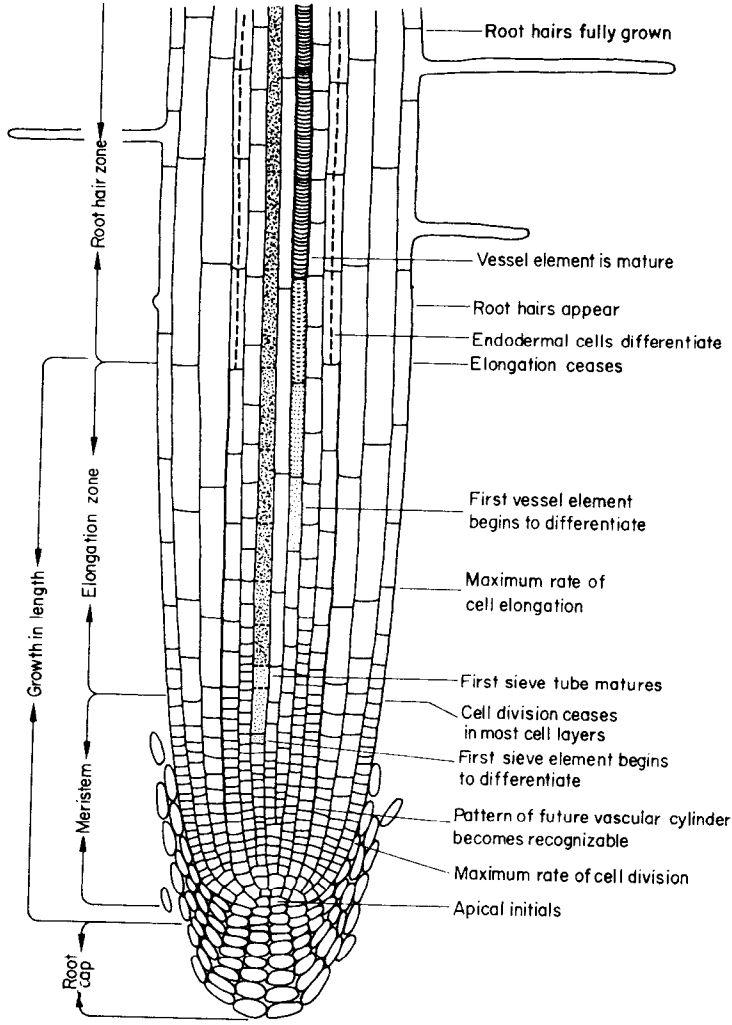

FIG. 1.1. Simplified diagram of the growing zone of a root, in longitudinal section. The number of cells in a living root is normally much greater than is shown in this diagram. (Reprinted from P. M. Ray, *The Living Plant*, Holt, Rinehart & Winston, New York and London, 1963.)

given by its water potential (ψ) which is equal to the osmotic potential (π) of the vacuolar solution plus the wall or turgor pressure (ρ). That is, $\psi = \rho + \pi$. Now clearly water uptake may involve changes in either the osmotic potential or in the wall pressure, or both. Studies on the changes in osmotic potential of the vacuolar solution during growth have yielded no evidence of a change in osmotic potential. Indeed, since the vacuolar sap becomes greatly diluted during growth, considerable amounts of additional osmotically active substances, such as sugars, salts, organic acids, etc., must pass into the vacuole during growth, in order simply to maintain the osmotic potential at a steady value. In some organs the osmotic potential of the vacuole may actually rise during this phase of growth. Thus, in the petioles of the water lily, *Victoria regia*, which may increase in length from 9 cm to 68 cm in 24 hours, the osmotic value may rise to less than half its original value during the extension phase. On the other hand, there is considerable evidence that in vacuolating cells the wall pressure is reduced by increased plasticity of the cell wall at this time (p. 83). As a result of its increased plastic extensibility the wall undergoes irreversible elongation during vacuolation.

Although the greater part of the increase in cell volume during vacuolation is due to water uptake, the synthesis of new cytoplasm and cell wall material proceeds actively during this period, so that the cell increases considerably in dry weight (Fig. 1.2). Thus, the

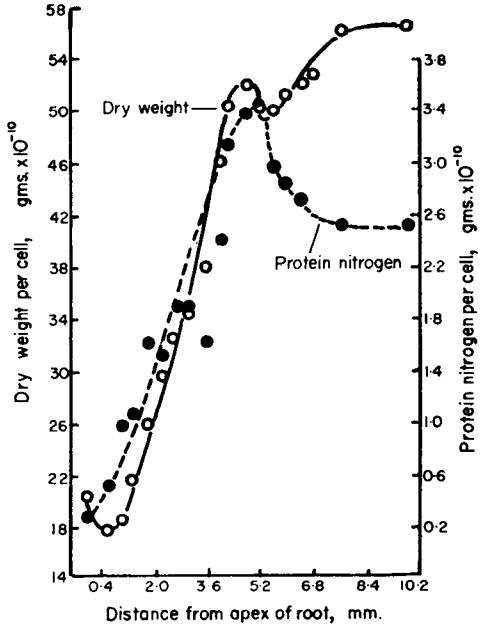

FIG. 1.2. Changes in dry weight and protein content of cells at increasing distances from the apex of pea roots. (Adapted from R. Brown and D. Broadbent, *J. Exp. Bot.* **1**, 249–63, 1950.)

processes of cell growth initiated before vacuolation commences are continued during this latter phase. Moreover, the zones of cell division and cell vacuolation are not sharply demarcated, and in both shoots and roots of many species cell division occurs in cells which have started to undergo considerable vacuolation (Fig. 1.3). Division may also occur in vacuolated cells in wound tissues. In root tips the separation of the zones of division and vacuolation are somewhat sharper and division in vacuolated cells is less frequent.

Since growth involves various endergonic, i.e. energy-requiring processes, including protein synthesis, it is not surprising to find that rapidly elongating tissues of the root have a high respiration rate, when compared with mature tissues on the basis of equal *volumes* of tissue, although when expressed *per cell* the respiration rate of mature cells may be greater than that of meristematic cells, since the latter are smaller and contain less cytoplasm. Moreover, growth requires aerobic conditions and an adequate supply of carbohydrate, both as an energy source and as structural material.

The role of growth hormones in cell division and cell extension will be discussed later.

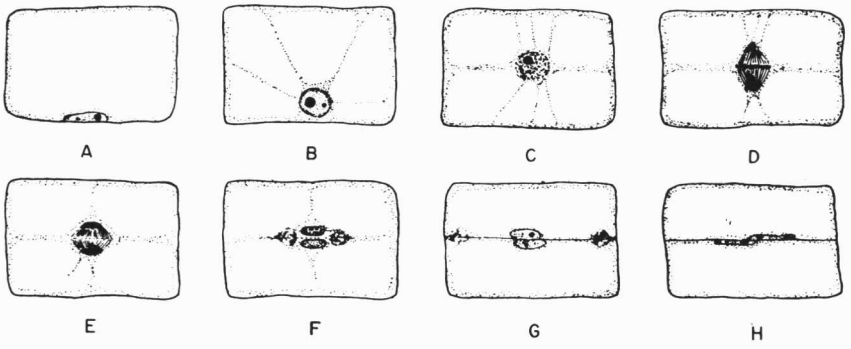

FIG. 1.3. Cell division in vacuolated cells. A, interphase; B, early prophase; C, prophase; D, metaphase; E, anaphase; F and G, telophases; H, two daughter cells at interphase. (From E. W. Sinnott and R. Bloch, *Amer. J. Bot.* **28,** 1941.)

GROWTH OF CELL WALLS

During cell extension the area of the cell wall may increase greatly and this fact poses a number of problems. It might be expected that as the wall is stretched by turgor pressure, it would decrease in thickness, but usually this does not occur. Hence, new material must be added to the wall during growth. There has long been a dispute as to whether the new material is added by "intussusception" throughout the thickness of the wall, or whether it is added to the interior surface, i.e. by "apposition". The bulk of the evidence now supports the second view, at least for many types of cell, but the possibility that there is also some intussusception cannot be excluded. Before we can consider the problem of wall growth further, however, it is necessary to consider the structure of the wall.

Electron microscopic studies have shown that the main structural element of the wall in higher plants consists of a framework of cellulose *microfibrils* (Fig. 1.4), which are somewhat flattened in cross-section, having a width of 10–30 nm,† a thickness of 5–10 nm and a length of at least 60 nm. The cellulose of the microfibrils is mainly present in a crystalline state, i.e. the molecules are regularly arranged in a lattice, while the remainder is semi- or para-crystalline. The microfibrils are embedded in a continuous matrix, consisting mainly of the so called "*hemicelluloses*" (non-cellulosic polysaccharides, composed mainly of residues of the pentoses, arabinose and xylose, and the hexoses, glucose, galactose and mannose) and "*pectins*", which contain a high proportion of galacturonic acid residues. The matrix also contains low amounts of proteins and lipids. (Further details of the composition of cell walls are given in Chapter 4, pp. 84–85.)

Growth of the wall involves the yielding of the wall to the stress generated by turgor pressure. During the extension of the walls the microfibrils become reoriented. In a typical parenchymatous cell undergoing elongation, the microfibrils are at first oriented in a transverse direction (i.e. at right angles to the long axis of the cell), but as the wall becomes stretched they may be arranged predominantly along the longitudinal axis. During growth, however, new transverse microfibrils are added to the inside of the wall, so that in a cross-section of the wall we find a gradual transition from transversely to longitudinally oriented microfibrils, in passing from the inside to the outside (Fig. 1.5).

The increased plasticity of the cell wall during vacuolation, referred to earlier, must indicate that the various types of chemical bond which link the different wall components must be broken during wall growth, possibly as the result of the activities of hydrolytic enzymes (p. 87).

In many types of cells, growth occurs fairly uniformly over the whole wall, giving the so-called "multi-net" pattern of growth. In other cases, as in root hairs and pollen tubes, the cell may extend by "tip growth"; in such cases it is found that in the growing tip region of a cell the microfibrils are oriented in a random fashion (Fig. 1.4), but during the process of wall stretching they become predominantly oriented in the direction of the cell axis. (Figure 1.4 relates to the primary wall of the alga, *Valonia*, but a similar structure is found in the tip region of cells which show tip growth.)

It is not known what determines the initial transverse arrangement of the microfibrils, but it is found that they usually lie parallel to certain *microtubules* which, as the name suggests, are elongated cylindrical structures of diameter 23–27 nm, found in the boundary layers of the cytoplasm (Fig. 1.6). Moreover, treatment with colchicine, which disrupts the microtubules, also disorganizes the arrangement of the microfibrils, but does not prevent their deposition. Thus, the orientation of the microtubules may, in some way not yet understood, determine that of the microfibrils of the wall.

The Golgi bodies also appear to play a role in cell wall synthesis, since they are conspicuous in regions of active wall synthesis, especially during the development of the cell plate following division (see below). Moreover, vesicles formed by the Golgi bodies have been

† Nanometers = 1 m $\times$ 10^{-9}.

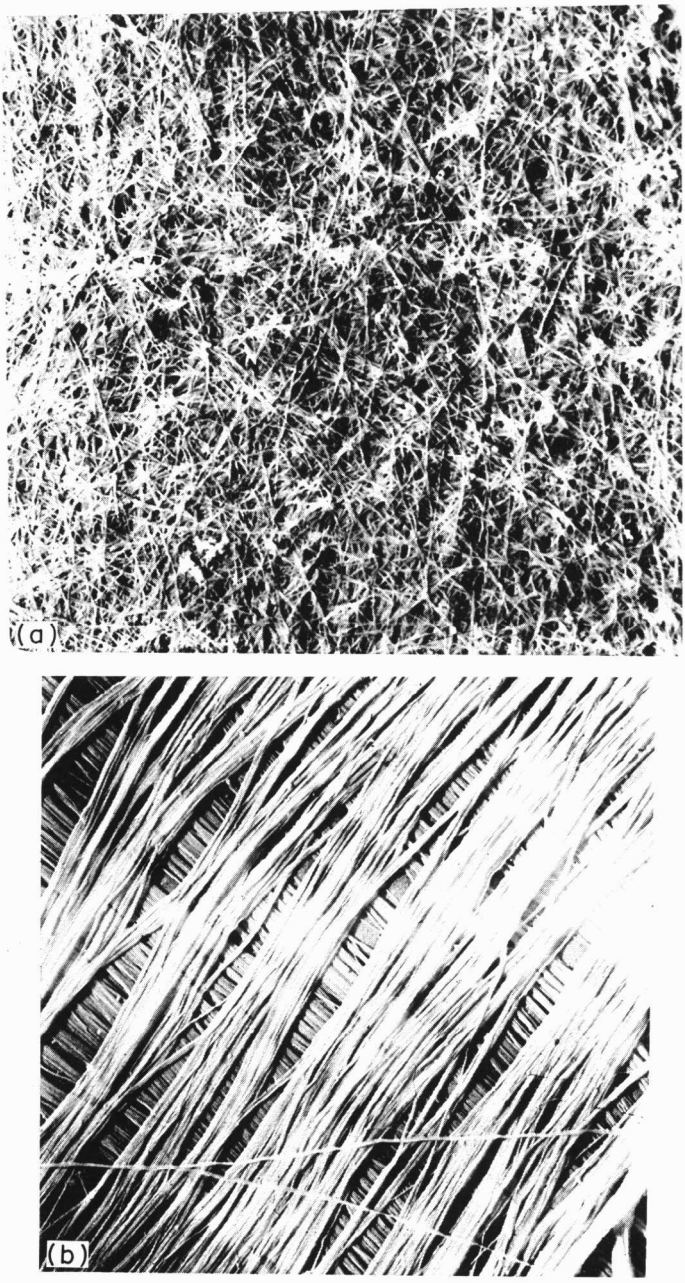

FIG. 1.4. (a) Electron micrograph showing structure of the primary wall of *Valonia* ×8000. (b) As above, but of the secondary wall ×7000. (From F. C. Steward and K. Mühlethaler, *Ann. Bot.* N.S., **17**, 295, 1953.)