PLANTS WITHOUT CELLULOSE

It was once believed that cellulose was the structural material in the cell walls of all green plants. Now it appears that other substances are employed for this purpose in certain marine algae

by R. D. Preston

Until quite recently the statement that all green plants contain cellulose seemed about as correct as the statement that all vertebrates contain bone. Cellulose is the structural constituent of the walls of the cells of green plants; it is a basically fibrous substance that gives the cell walls form and rigidity. Wood is about 40 percent cellulose. Cotton, linen and the fibrous products made from wood pulp—paper, rayon and so on—consist of little else.

It has therefore been startling to discover that there are two groups of green plants in which the cell walls are based not on cellulose but on two quite different substances. In one of these substances the molecular subunits are linked together in a way that is different from the linkages in cellulose; hence the molecules pack together differently in the fibrils, the structural units of the cell wall. In the other substance the linkages are the same as those in cellulose but the substance seems to form fibrils only occasionally.

The first indication that green plants lacking cellulose might exist came some time ago, when it proved impossible to demonstrate the presence of cellulose in certain plants. These demonstrations relied, however, on staining methods, which are notoriously unreliable. Consequently the cell walls of the anomalous plants did not attract much attention. Then about five years ago, by one of those coincidences that come about in science presumably because the atmosphere is right, several laboratories in different parts of the world independently began to study these odd plants. One of the laboratories was our own at the University of Leeds. Using approaches that were not available to the earlier workers, we have shown that such plants do indeed lack cellulose, and we have gone on to explore their molecular architecture.

The problem of how the cell wall of plants is built is an important one even apart from the fact that such essential materials as wood, cotton and paper are virtually cell walls in their natural state. The problem is important because the cell wall forms a rigid or semirigid envelope around each living unit in all plants. A plant can grow only at such a rate and in such a way as its constituent cells will allow; their growth is in turn restricted by the physical properties of their walls, and these properties of course depend on the walls' detailed molecular structure. Accordingly the shape of a plant and the rate at which it reaches its final form can in principle be traced back to the structure of the cell wall.

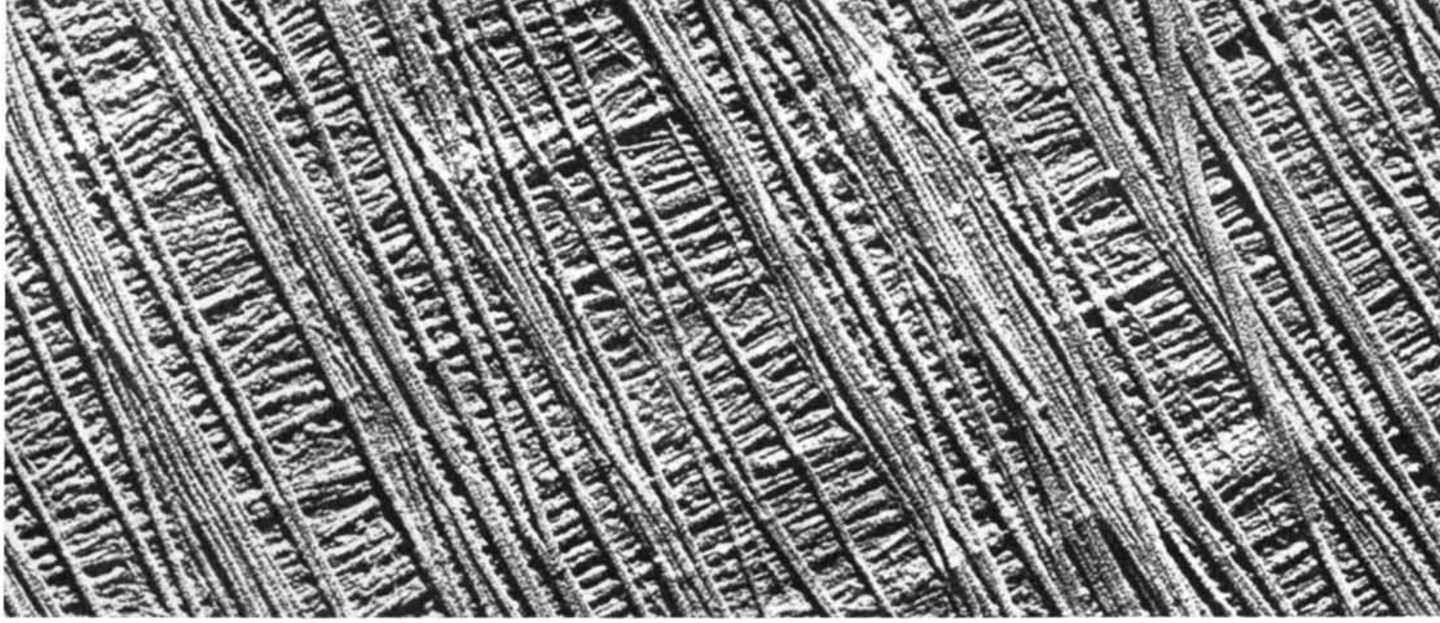
All the plants that lack cellulose are algae, and most of them are inhabitants of the warmer seas. They are interesting in their own right. Some of them, in spite of the fact that their cellular construction is comparatively simple, have achieved spectacular forms not surpassed by higher plants [*see illustration on page 104*]. One of them, *Acetabularia,* is widely used as a model cell in studies of the relation between the nucleus and the surrounding cytoplasm.

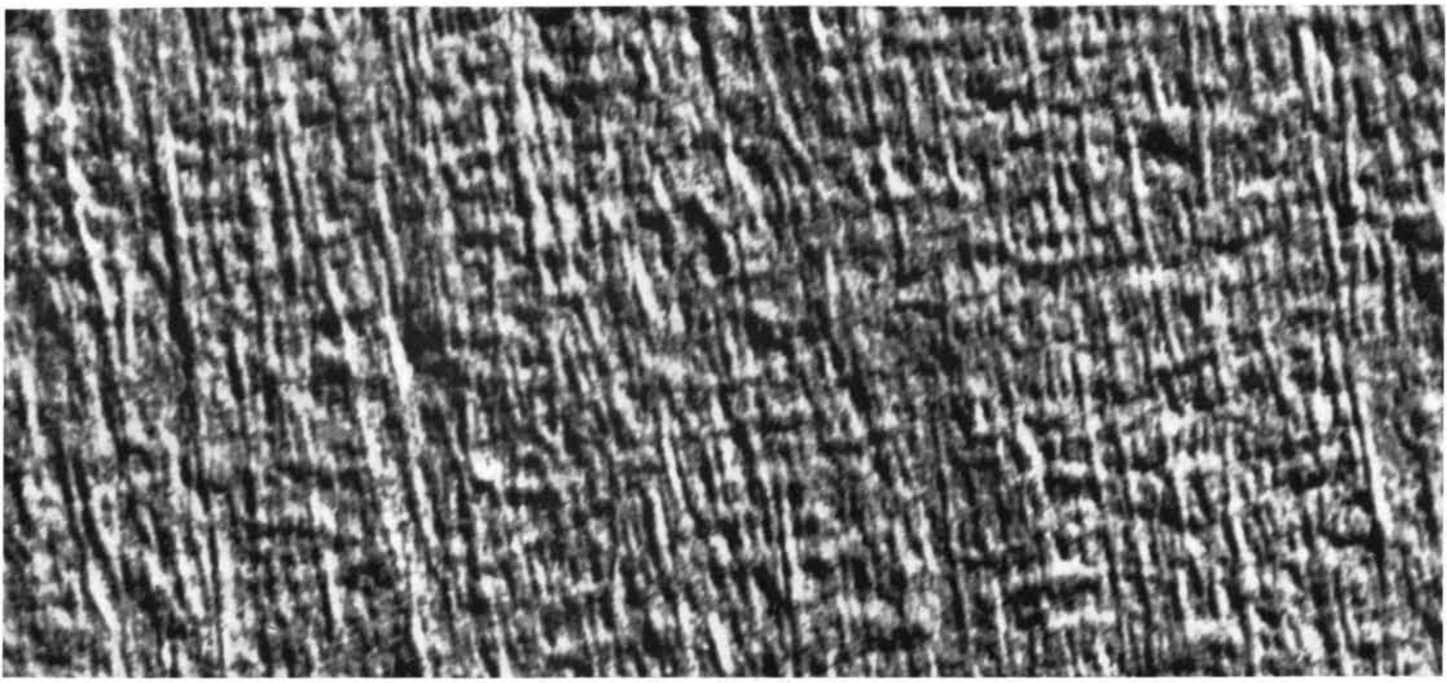
As a group these seaweeds have considerably broadened our understanding of the structure of plant cell walls. Since their cell walls are different from those of all other green plants, they broaden the range of possible structures affecting plant growth, offering a basis against which we can assess knowledge obtained from cellulosic plants and thus define the factors involved in plant growth more sharply.

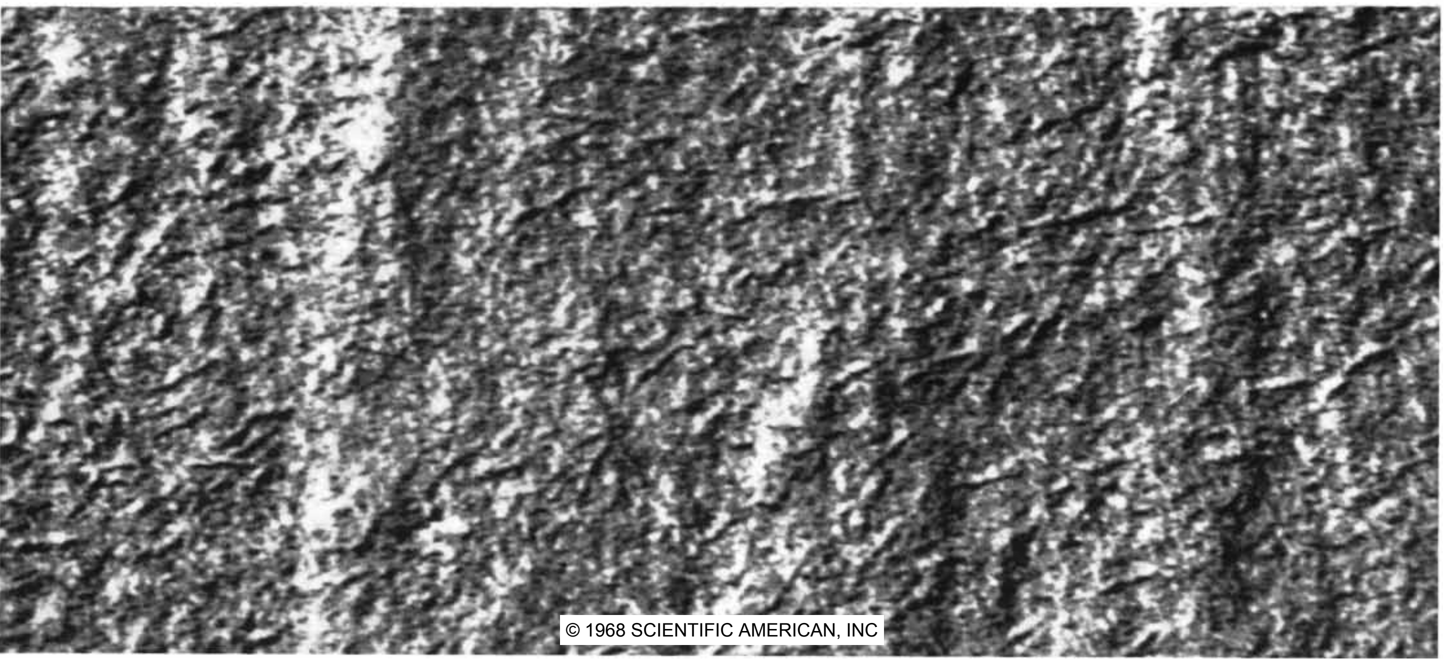
To understand how differently the walls of the noncellulosic plants are constructed, let us first consider the cell walls that contain cellulose. It is convenient, if somewhat oversimplified, to regard the cell wall as having two components. One of these components consists of partially crystalline microfibrils, that is, thin threads partly made up of long-chain molecules packed together in a regular parallel array. The microfibrils are between 100 and 200 angstrom units wide, about half as thick and so long as to be virtually endless. They are embedded in the second component of the cell wall, a less crystalline cementing matrix. The regular parallel arrays within the microfibrils are cellulose; the cementing matrix consists of substances that, like cellulose, are polysaccharides but are made up of different subunits.

The subunits of cellulose are the simple ring-shaped structures of the sugar glucose. The ring includes five carbon atoms, each of which is identified by number [*see illustration on page 105*]. When one glucose molecule is linked to another, the carbon atom designated 1 is joined through an oxygen atom to carbon atom 4 of the other molecule. At either end of the dimer thus formed another glucose molecule can be attached in the

DIVERGENT STRUCTURE of the cell wall of plants is revealed in electron micrographs on opposite page. At top the tiny threads, or microfibrils, of the marine alga *Chaetomorpha melagonium* show distinctly. The microfibrils are composed of cellulose, as are those of all known higher plants. In the middle is a comparably magnified sample of the green alga *Penicillus dumetosus.* Short crossbars appear to connect the vertically running microfibrils. Although in other respects the fibrils resemble those in the micrograph at top, they are not made of cellulose. At bottom is the cell wall of the alga *Batophora,* which also lacks cellulose. No microfibrils are visible; instead the wall appears to consist mainly of granules. Magnification of the micrographs (from top to bottom) is 65,000, 75,000 and 100,000 diameters.







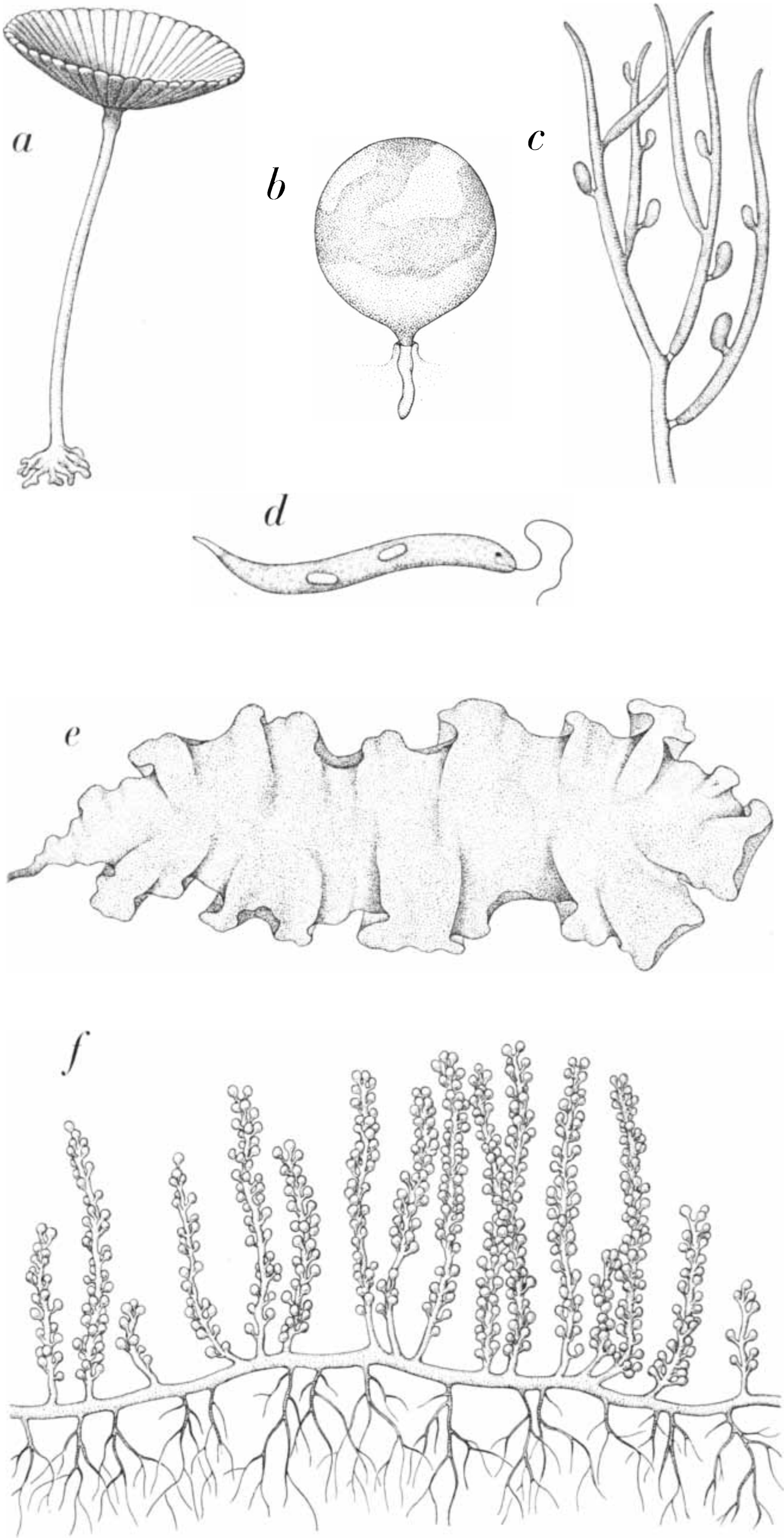
same way. If the process is continued, it ultimately gives rise to cellulose: a chain of perhaps 10,000 or 15,000 glucose units. The chain appears to be straight rather than coiled or folded (folded structures have been suggested but the evidence is not convincing), probably because of certain hydrogen bonds between its subunits. These straight chains, lying parallel to one another for at least part of their length, form a crystalline core in the microfibril.

The cementing matrix consists of such polysaccharides as xylan, mannan, araban, galactan, glucomannan and polygalacturonic acid. The subunits of these polysaccharides are sugars other than glucose. The molecular chains of matrix polysaccharides lie parallel to the microfibrils but are irregularly arranged and therefore only slightly crystalline. The cell wall owes its coherence to bonding between these amorphous substances and the microfibrils: hydrogen bonds, bridges through calcium and magnesium ions and, it is now believed, linkages to a specific protein found in cell walls.

This, then, is the general structure of the cell wall of the plants that contain cellulose. Let us now examine our anomalous algae. They are divided into two groups. Both were at one time classified in a single order, the Siphonales (a term that has since been dropped because it was not based on a legitimate family name). In one group cellulose is replaced by xylan, a polymer, or repeating chain, of the pentose sugar xylose; in the other group it is replaced by mannan, a polymer of the hexose sugar mannose. It will be convenient to examine the xylan seaweeds first.

All these plants are of filamentous construction. They range from a single-branched filament in *Bryopsis,* through an intricately formed filament simulating the habit of higher plants in *Caulerpa,* to a compact body of associated filaments in *Halimeda* or *Penicillus.* When subjected to X-ray analysis, the xylan plants all display the same diffraction pattern; it is markedly unlike the pattern obtained from cellulose. When the crystalline wall material of such plants is decomposed, it yields only xylose, indicating that the material is certainly a xylan.

The structure of the xylose molecule is much like that of the glucose molecule except that the sixth carbon atom, with its associated groups, is replaced by hydrogen. In the xylans found in the wall matrix of the cellulose-containing higher plants the xyloses are joined carbon 1 to 4, like the glucoses in cellulose. On the other hand, the X-ray pattern of the sea-



EXCEPTIONAL MARINE PLANTS differ from other green plants in cell structure. The single-celled *Acetabularia* (*a*) has its nucleus in the "stem," making it a useful model in cell studies. *Halicystis* (*b*) and *Derbesia* (*c*) are two generations of the same plant. The divergent feature in the structure of *Euglena* (*d*) is the paramylum, or food reserve, forming two lozenge-shaped bodies. *Porphyra* (*e*) is a papery red seaweed. *Caulerpa* (*f*) has structures like roots and branches but is a single cell. The plants shown here are not drawn to scale.

weed xylans is different from the pattern of higher-plant xylans; hence the seaweed xylan must be built up some other way. The only link possible is between carbon atoms 1 and 3.

This was the conclusion to which our studies led. It would perhaps have been the first demonstration of a link between the sugars of a polysaccharide by X-ray analysis alone. Before Eva Frei and I published our findings, however, 1,3-linked xylan had been detected in some of the seaweeds by chemical methods, first by I. M. Mackie and Elizabeth Percival of the University of Edinburgh and then by Y. Iriki and T. Miwa in Japan.

When the subunits of xylan are linked carbon 1 to carbon 4, they form a straight chain (although, as has been shown by Robert H. Marchessault of the State University of New York College of Forestry at Syracuse University, the chain is probably twisted around its long axis). With a 1,3 linkage the chain can only be curved. Nevertheless, in the electron microscope the fibrils of seaweed xylan are reasonably straight. In what way can curved chains pack together to form straight fibrils? One possibility is that the chains are coiled into straight helices. The helices would need to be fairly flat, because observations with the polarizing microscope show that the individual chains lie more nearly at right angles to the microfibrils than parallel to them. Evidence provided by X-ray analysis led us to conclude in 1964 that within a crystalline xylan fibril the chains are coiled in double helices. On the basis of these and other findings we worked out at that time a model of the xylan fibril.

Recently two of my colleagues, K. D. Parker and E. D. T. Atkins, have refined this model by applying helical diffraction theory to the X-ray diagram and by the use of polarized infrared spectrophotometry. They have demonstrated that three chains, not two, are coiled together, stabilized by interchain hydrogen bonding [*see bottom illustration on next page*]. At the moment it seems likely that the chains are coiled in a right-handed sense. We believe this represents an exceptionally precise determination of structure for a polysaccharide.

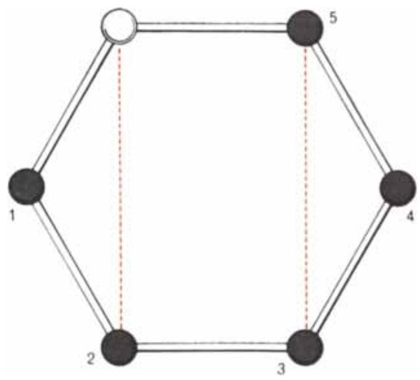
It is interesting that a specimen of xylan is more crystalline when it is wet than when it is dry; water is required (to the extent of 30 percent of the weight) if the chains are to lie in perfect order. This is quite different from the behavior of cellulose, which is equally crystalline dry or wet, water being unable to penetrate the crystal structure. It appears that insofar as crystallinity is indispensable to a structural polysaccharide, 1,3-linked xylan is better suited to water plants than to plants on dry land. This may be why land plants have not, as far as we know, used the substance as a supporting framework for their cells.

Although the xylan seaweeds use xylose in preference to glucose as a structural subunit, glucose does appear among the polysaccharides that form the matrix substance of their walls. Thus the roles of the two sugars in the xylan seaweeds and higher plants are reversed. It has been reported that the glucose units in the walls of the seaweeds are 1,3-linked. It is not clear to what extent the reversed roles of glucose and xylose are related to the 1,3 linkage as against the 1,4 one.

A number of other polysaccharides spread through the plant kingdom are known to be 1,3-linked. We and others have examined some of them and we have found that their X-ray patterns are much alike and similar to that of the xylan. We are now looking into the possibility that most polysaccharides so linked—for example the laminarin of brown seaweeds, the paramylum of *Euglena* and the callose of higher plants—also form helices. It is quite likely that the most abundant polysaccharides on the earth are not, as we once thought, the straight-chain 1,4-linked polysaccharides cellulose and starch but rather the polysaccharides that are 1,3-linked and helical. These substances can be expected to have mechanical properties quite different from those of the straight-chain ones.

The second group of plants I wish to examine here is unusual in quite a different way. These plants, which include *Acetabularia*, are again basically filamentous. The only crystalline polysaccharide present in their walls is mannan. It is identical with the mannan found in higher plants. Mannose, the sugar of which mannan is composed, closely resembles glucose; the molecular structure of the two sugars differs only in the reversed positions of a hydroxyl group (OH) and a hydrogen on one of the carbons in the ring. Like cellulose, the units of mannan are linked through carbon 1 to carbon 4.

Although mannan is common among higher plants, it is detectable as a crystalline polysaccharide only in the seeds of one group of them. Even there it is not the only structural polysaccharide, the cell walls of the plants being basically cellulosic. This group is the palms, among them the date and the ivory-nut palm (the toughness of mannan is indicated by the name and the fact that laboratory-coat buttons are sometimes still



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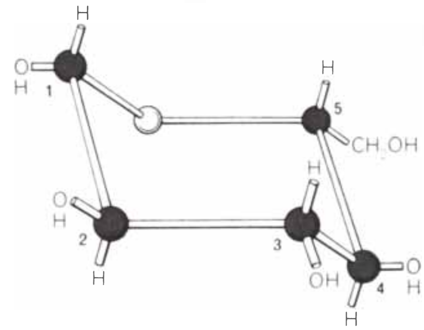
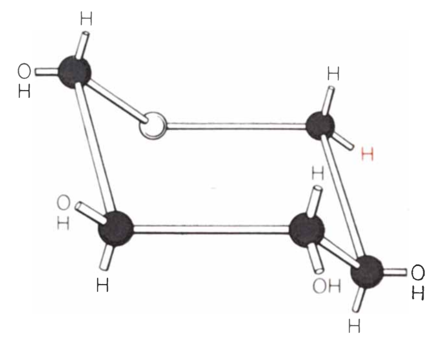
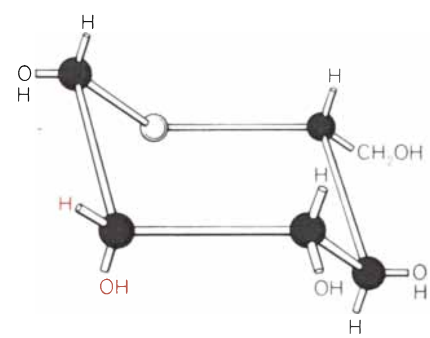


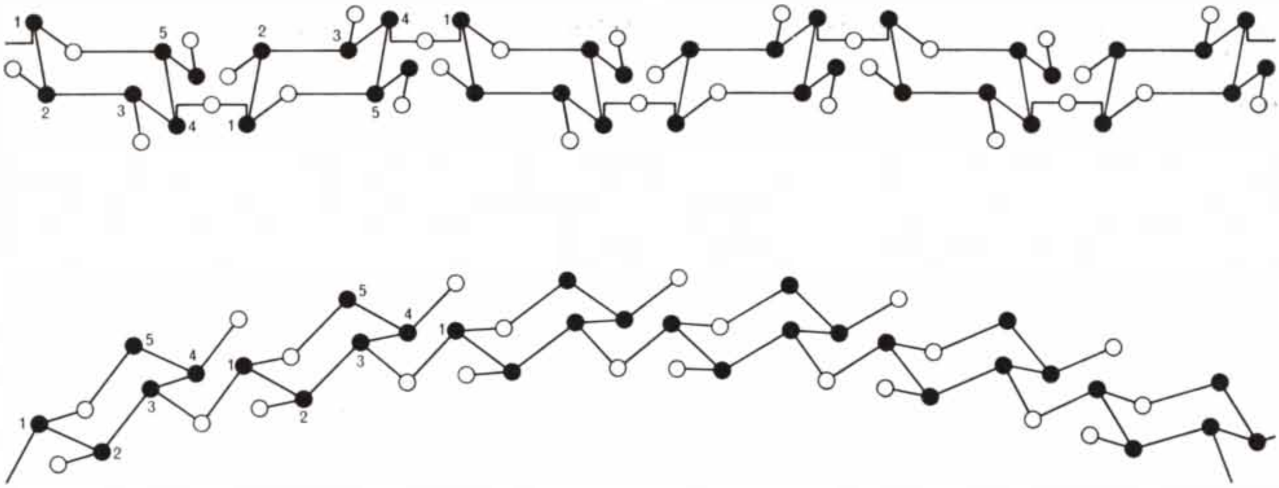
XYLOSE



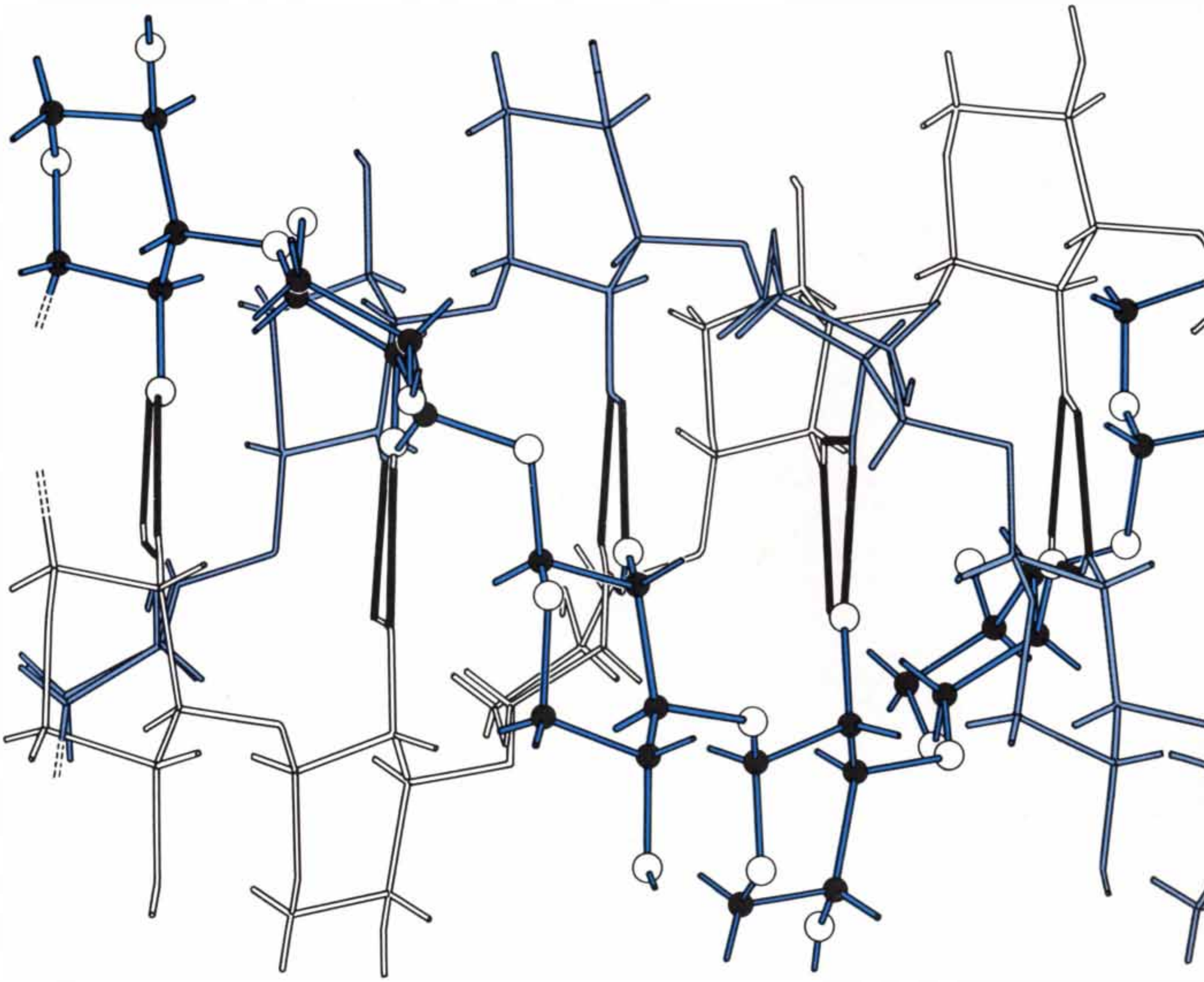
MANNOSE



MOLECULAR DIAGRAMS indicate (*color*) where xylose and mannose differ from glucose. All these sugars have a hexagonal ring (*top*) composed of carbon atoms (*black balls*) and an oxygen atom (*white ball*). In the diagrams this hexagon is seen from the edge with two corners bent. Cellulose is formed from glucose; xylose and mannose form the substances that replace cellulose.

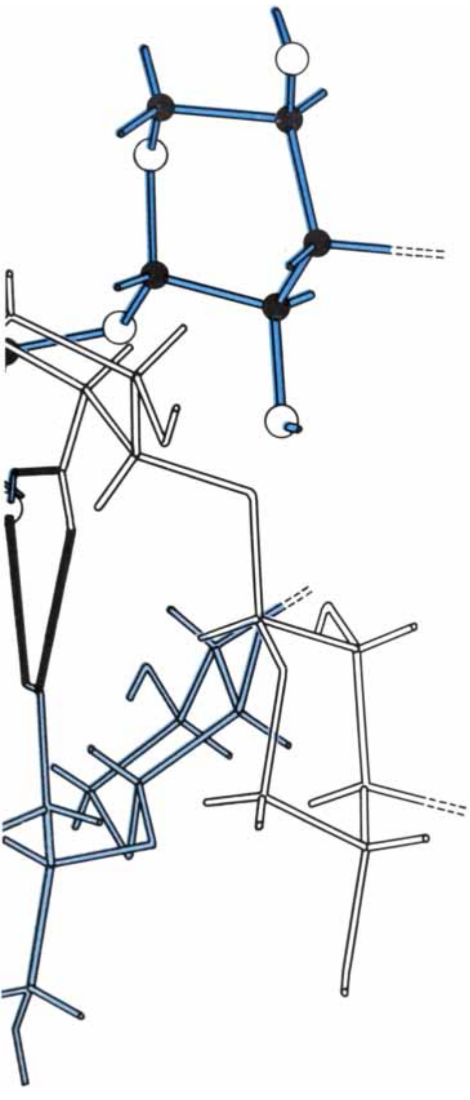


MOLECULAR CHAINS are linked through oxygen atoms. A short segment of the cellulose chain is shown at top with carbon atoms in the hexagonal ring of some of the units numbered. The linking in the chain of carbon atom 1 and carbon atom 4 gives rise to a regular alternation in the orientation of the glucose units and a straight chain. Below the cellulose chain appears the segment of a xylan chain that is composed of xylose units. The units are joined at carbon atoms 1 and 3, and this linkage produces a curved chain.



TWISTING OF XYLAN CHAINS gives rise to a straight microfibril. Each straight section of the three chains forming the helix represents a bond between atoms; the black linkages are hydrogen bonds that stabilize the chains. The chains are shown as being coiled in a right-handed sense; in one chain oxygen and carbon atoms are depicted. The drawing is based on a model worked out made of "vegetable ivory" in spite of the availability of plastics).

Through X-ray analysis of the cell walls of mannan seaweeds it has been possible for the first time to provisionally elucidate the crystal structure of mannan. It appears to resemble the crystal structure of cellulose in that the chains of mannose units lie parallel to one another; it is dissimilar in that the chains pack together somewhat differently. Neither fact is surprising in view of the specific difference between the molecules of mannose and glucose. The molecular chains of mannan are commonly reported to be much shorter than those of cellulose, ranging up to 80 mannose units. My colleagues W. Mackie and D. B. Sellen have given reason to believe, however, that the chains of seaweed mannan approach the length of cellulose chains.



by the author and his colleagues at the University of Leeds. Hydrogen atoms are omitted from this illustration and the one at top.

The surprise comes when one examines under the electron microscope a sample of the cell-wall material that attests (on the basis of X-ray analysis) to mannan's crystal structure. One would expect to find much the same kind of well-oriented fibril that one sees in a comparable micrograph of cellulose or xylan. In spite of the most determined efforts such fibrils have not been detected by the methods that succeed with cellulose and xylan seaweeds. These methods involve the removal of the noncellulosic polysaccharides by chemical means. The polysaccharides that surround the crystalline regions of mannan, however, are themselves mostly mannan; they cannot be removed to reveal a possible underlying fibril structure without the risk of destroying the structure. Although the microfibrils of cellulose and xylan are often visible without the removal of the surrounding matrix, it is still not safe to conclude that in mannan walls microfibrils are completely absent. In fact, Mackie has shown recently that exceptionally mild treatment of mannan walls reveals occasional microfibrils. Nevertheless, in electron micrographs seaweed mannan appears most often to take the form of granules or at most very short rods (no longer than 2,000 angstroms). The granules must be mutually oriented in an orderly fashion to provide the observed X-ray pattern.

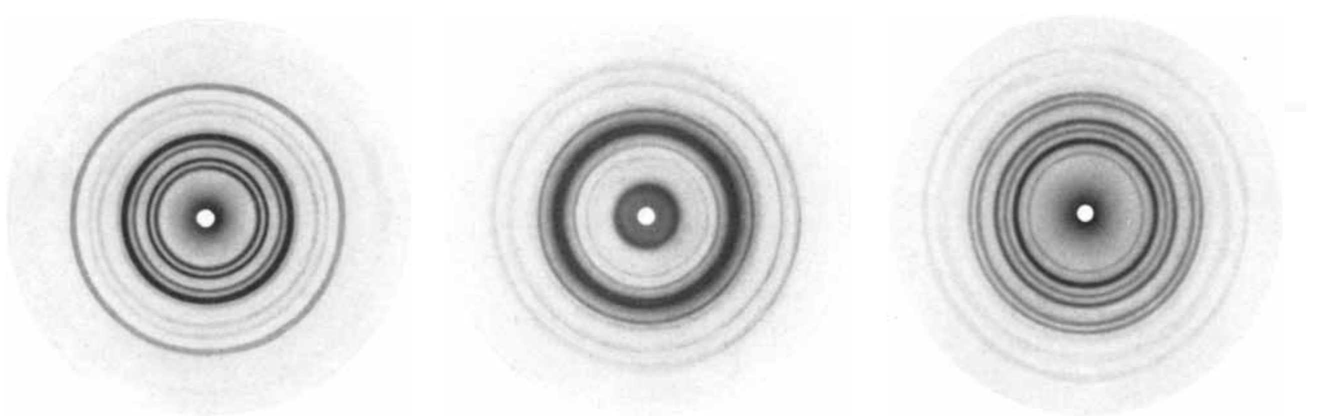
This presents problems even though microfibrils are occasionally present. If one assumes that the mechanical properties of the mannan walls are not much different from those of cellulose walls (this assumption is supported by manipulations of the walls), how does it come about that an array of granules behaves like an array of parallel threads? What mechanism specifies the particular order the granules display? It is possible to conceive of a mechanism by which threads can be arranged parallel to one another, and I have already proposed such a mechanism in general terms. It is much more difficult to imagine a mutual orientation of granules under the conditions that are known to exist at the cell surface.

In searching for an explanation we must bear in mind that the cell-wall layers examined in the electron microscope have been subjected to drastic drying in the process. Furthermore, under these conditions a structural element with one dimension of less than eight angstroms is not visible at all in the kind of material involved. One could therefore imagine the wall to be a parallel array of short molecular chains that overlap one another and that in small granule-shaped regions, and in these regions only, are regularly spaced and therefore crystalline. Such a structure could give rise to both our X-ray patterns and our electron micrographs, and would be in agreement with the chain lengths found by Mackie and Sellen.

The mannan cell walls appear to have significance for certain theories of plant growth. The growth rate of plant cells is known to be under the control of auxins, or plant hormones. The view most widely held today is that the auxins achieve their effect by changing the coherence of the cell wall. The cell-wall components believed until recently to be the most intimately involved are the pectic compounds, derivatives of polygalacturonic acid that are found in the matrix substances of all higher plants.

Now, the auxins are also said to affect the development of the seaweed *Acetabularia*. The wall of this plant is lacking not only in cellulose but also—according to our analyses and those of other laboratories—in pectic compounds. We must therefore assume either that this plant is peculiar in still another way or that the wall substance affected by the auxins is not polygalacturonic acid. The latter seems to me the more likely, particularly in view of work that points in quite another direction. One of my students, E. W. Thompson, has detected in the walls of a noncellulosic plant (*Codium tomentosum*, which contains mannan) an abundance of a specific protein that D. A. Lamport of Michigan State University has shown to be present in the walls of higher plants. Lamport gives reason to believe the protein is a wall component closely associated with cell-wall extensibility, and we have reached the same conclusion both for mannan-containing and cellulose-containing seaweeds. This may prove not to be the whole story, but our work implies that it is at least an important part of it.

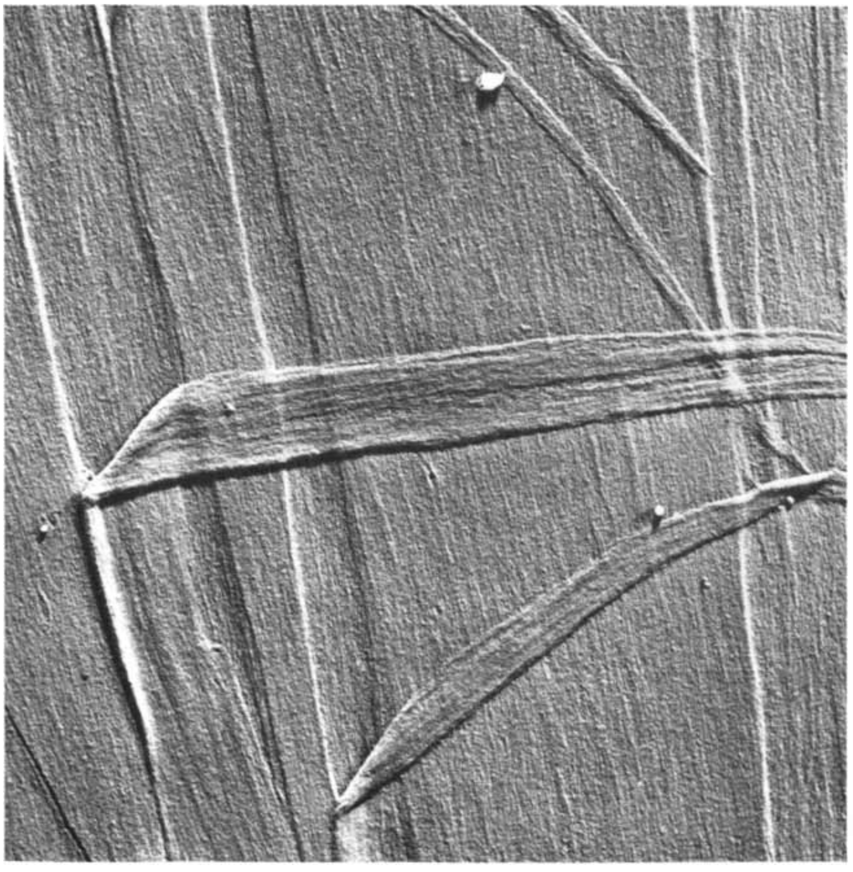
When we first began our study of these odd plants, we suspected that (to argue teleologically) plants in the sea might have experimented with a variety of polysaccharides as cell-wall skeletal materials. As it has turned out this suspicion was justified. It remains to be seen what other surprises may await us among the lower plants. We know already of two other groups of plants, also seaweeds, that combine in themselves the structures found separately in the mannan and xylan algae. In one of these groups the prominent member is *Porphyra*, familiar as the papery red seaweed that in season clothes many of the rocks along coasts in the Northern Hemi-



X-RAY DIFFRACTION PATTERNS indicate differences in the cell structure of seaweeds. At left is the pattern made by *Chaetomorpha*, whose cell walls contain cellulose. The middle pattern is of *Penicillus*, in which xylan replaces cellulose. At right is the pattern of *Batophora*, whose cell walls contain mannan, which is composed of mannose units and also substitutes for cellulose.

sphere. In this plant the cell walls contain crystalline 1,3-linked xylan, and the cuticle covering the walls contains crystalline 1,4-linked mannan.

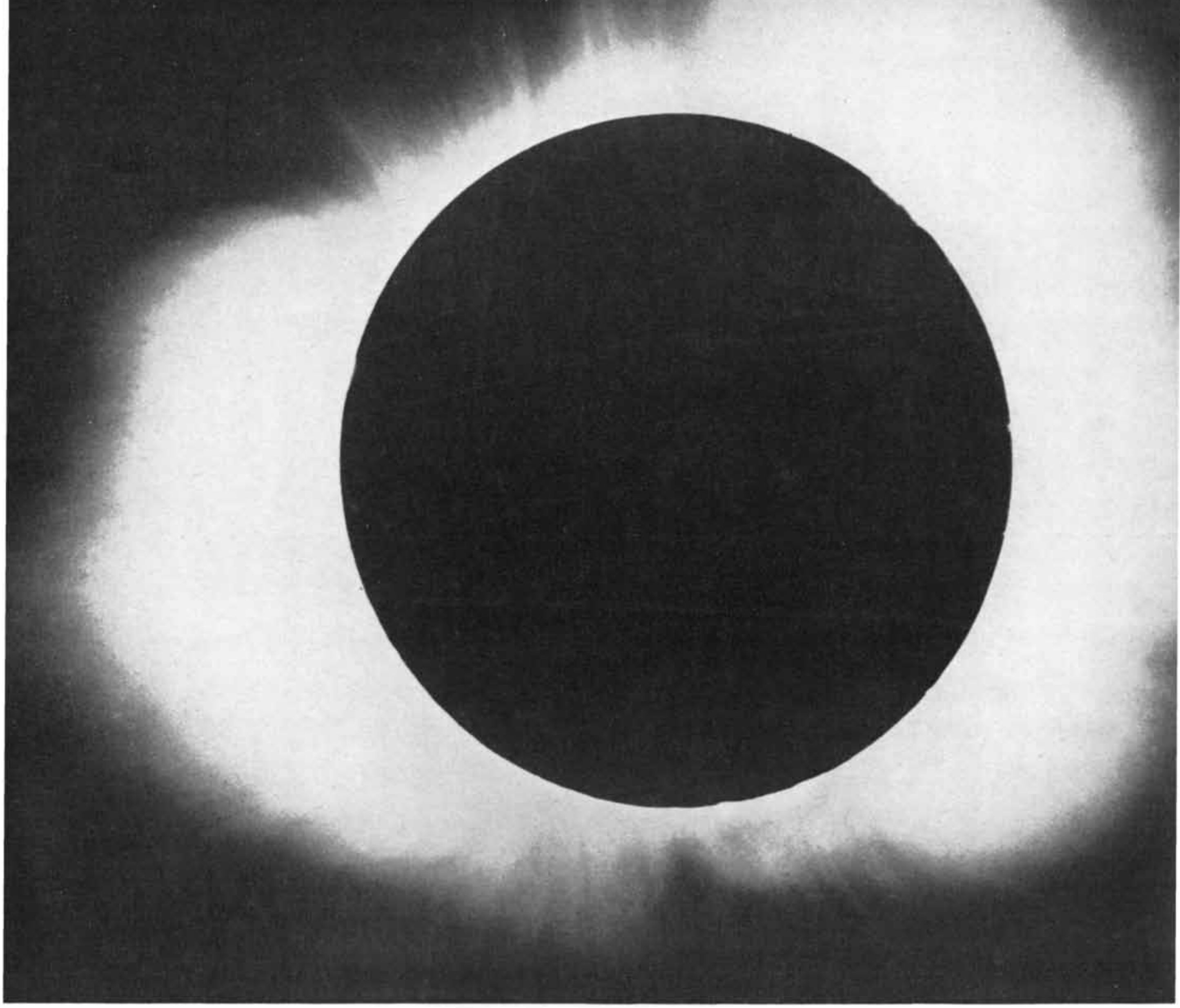
The other example of combined structure, involving the plant *Halicystis* (commonly known as sea bottle and consisting of single "cells" or vesicles as large as a pea), is somewhat more complicated. *Halicystis* is a haplont, which is to say that each of its nuclei has only a single set of chromosomes. During reproduction the nuclei fuse in pairs, and the resulting cell ultimately develops to form a plant body called the diplont generation. Eventually some or all of the nuclei of the diplont plant undergo the division known as meiosis, by which the chromosome number in each daughter nucleus is halved. The cells then divide by normal mitosis to reproduce the haplont plant body, and the cycle is repeated.

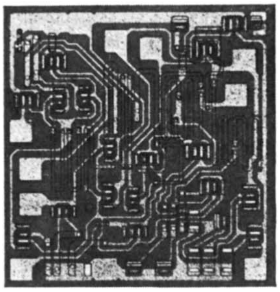


TEARING OF THE CELL WALL provides evidence of its underlying structure in the mannan seaweed *Dasycladus*. Like the cell wall of other mannan seaweeds, the wall of *Dasycladus* appears to be predominantly composed of granules. The fact that the specimen tears cleanly in a direction parallel to the grain of the wall indicates that the granules are in an ordered array. In this electron micrograph the specimen is enlarged about 6,500 diameters.

Now, although *Halicystis* is composed of roughly globular large cells, its diplont phase is finely filamentous; the diplont is known by the name *Derbesia*. *Halicystis* and *Derbesia* are two generations of one and the same plant. Nevertheless, the walls of *Halicystis* contain crystalline 1,3-linked xylan together with some cellulose but no mannan, and the walls of *Derbesia* contain crystalline mannan but neither cellulose nor 1,3-linked xylan. This is a clear case of a correlation between the chromosome complement of a cell and the constitution of its wall.

It is a rather odd circumstance that although water plants such as those discussed here must have lived for many millions of years with their peculiar walls, as far as we know none of these walls is represented among land plants. It would be too much to say that the structure of all land plants is known—there may be some surprises waiting in this realm as well. Nonetheless, the consensus is that all green land plants will prove to have cellulose walls. It appears that several polysaccharides and polysaccharide combinations can make a serviceable cell wall for water plants but only one kind of wall serves those plants that have managed to colonize dry land. All we can conclude is that plants on dry land need in their cell walls a balance between the rigidity necessary to hold the plant upright and an extensibility—perhaps even a controlled breakdown—that will allow the plant to grow. Perhaps this balance can be achieved only in a wall based on cellulose.





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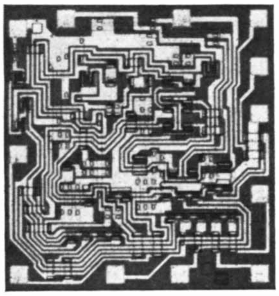

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