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The role of self-assembly in biological systems: evidence from iridescent colloidal sporopollenin in *Selaginella* megaspore walls

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[Plate 1]

SUMMARY

Iridescent exines have previously been reported in both fossil and modern megaspores of selaginellalean affinity. This striking feature has been shown to result from a colloidal crystal configuration of the exine units in at least part of the wall. This paper discusses the mechanics of formation of the colloidal crystal and of the adjacent wall layers (by depletion attraction), and provides a detailed explanation of the cause of the iridescence. *Selaginella* leaves and virus aggregates provide comparisons with the iridescent arrangement present in the spore wall.

The self-assembly aspect of colloidal structures in biological systems is explored. Periods of self-assembly in organismal development may represent a saving in terms of stored information retrieval. Self-assembly, and its often chaotic behaviour, offer the prospect of large changes in organismal construction associated with relatively little change in the underlying genetic configuration.

1. INTRODUCTION

Selaginella L. is a large genus of heterosporous lycophytes (e.g. figure 1), mostly tropical in distribution. In some species, a highly ordered layer occurs in the megaspore wall (Taylor 1991a; Hemsley *et al.* 1992). Spores with a similar layer are known as fossils (e.g. *Erlansonisporites* Potonié, *Thylakosporites* Potonié) with an age up to 80 Ma or more. Many other fossil spores exhibit a comparable but less regular organization (Taylor & Taylor 1988; Collinson *et al.* 1993; figures 17 and 19), and still more exhibit similar components but with no indication of regularity (Kempf 1971; Hemsley 1992; Kovach 1994). In those spores with regular layered structure, iridescence (flecks of colour) can be observed when the ordered layer is exposed (figures 4–6). Some species of *Selaginella* are also known to have leaves which exhibit iridescence (figures 2–3). This feature has been explained in detail by Héban & Lee (1984) and Lee (1986), and is known from other plant groups which, like the species of *Selaginella* that possess this feature, are adapted to a deep shade environment (Graham *et al.* 1993). Leaf iridescence in *Selaginella* is produced by a lamina of consistent thickness at the leaf surface (thin film iridescence), whereas spore iridescence is caused by a related but different

arrangement brought about by the presence of a crystalline colloid (Hemsley *et al.* 1992).

Colloids were suggested as being of relevance in the construction of complex biological systems as long ago as 1917 by Thompson (1961), who stated

‘... the phenomena with which we are about to deal go deeper into the subject of colloid chemistry, and especially that part of the science which deals with colloids in connection with surface phenomena. It is to the special student of the chemistry and physics of the colloids that we must look for the elucidation of our problem.’

Although he originally referred to the spicules of sponges and similar constructs, had Thompson been aware of the complex ultrastructure of spore and pollen exines, he would doubtless have considered them an analogous case.

In the intervening time, many studies have been undertaken on spore and pollen walls to elucidate their ultrastructure and development especially, recently, by using scanning (SEM) and transmission electron microscopy (TEM) (see, for example, Taylor 1991a). Much has been achieved in this time, particularly in terms of the expansion of our awareness of the diversity of exine ultrastructure in both living and fossil spores and pollen. Studies of

development have provided an insight into the mode of sporopollenin deposition in many living species, for example the complex networks of sporopollenin fibres illustrated by Rowley *et al.* (1981) and Rowley & Srivastava (1986), and the semi-regular polygonal base units observed by Kedves *et al.* (1991, 1993). However, as recently stated by Dickinson & Sheldon (1990), the precise way in which an exine appears to be constructed often seems peculiar to the group in question and, as a result, we are perhaps only slightly closer to a set of general principles of exine construction. In many cases it remains unclear how complex, often regular exine ultrastructure and surface ornament are produced. As with many problems, adopting an alternative viewpoint can be both pertinent and revealing; this is what we believe the application of colloidal theory can achieve in this instance.

Few studies of spore and pollen wall structure have sought an entirely new approach to the interpretation of ultrastructure and pattern development. Heslop-Harrison (1972) offers a cautious suggestion of self-assembly stating that

‘... control must be exerted only at certain strategic points, in determining what shall associate with what, and in what order, and in mapping out, in interaction with the cell environment, where the greatest probabilities will be for association to begin. After this, physical processes akin to crystallisation, or the formation of Lissajou figures, must take over to complete the “space-filling” operation.’

Van Uffelen (1991) also recognized the role of ‘non-biological’ processes in the formation of fern spore patterning. Her observations consisted of a number of comparisons between fern spore perine surface ornament and the patterns formed when glycerine jelly dries between slide and cover slip. She rightly referred to this as a physical process (condensation), independent of external influences. Despite this clear analogy, the idea that complexity and order could result from relatively simple physical and chemical processes had still not permeated palynological studies to any great extent.

In their reinvestigation of the complex *Selaginella* megaspore wall ultrastructure, Taylor & Taylor (1987) referred to the similarity of the iridescent spore walls to the appearance of precious opal, as had Hueber (1982), but neither pursued this observation. It is of note that the precious opal is itself a colloidal system and one which, in many ways, is comparable to the most ordered layer seen in the spore walls. It is this layer which has provided the clue to the process of formation of the exine as a whole.

A critical analysis of the highly ordered layer within *Selaginella* spore walls demonstrated that the previous model of construction (plates with regularly arranged peg-like projections (Taylor & Taylor 1987) implied the existence of features not encountered in any examination so far documented (see Hemsley *et al.* 1992). Instead, a simple arrangement of close-packed, more or less spherical particles was offered as the most likely construction (figures 7 and 8). Despite this change in concept regarding the basic unit of

Figure 1. *Selaginella* sp. exhibiting typical features of this genus. Both micro- and megaspores are borne in terminal cones consisting of a number of sporangia. Generally megasporangia are located toward the base of the cones. Scale bar = 20 mm.

Figure 2. *Selaginella* sp. exhibiting leaf iridescence in the blue–green range. Scale bar = 30 mm.

Figure 3. A different *Selaginella* exhibiting leaf iridescence at a shorter wavelength. Scale bar = 30 mm.

Figure 4. *Erlansonisporites* sp. (fossil *Selaginella* megaspore) illustrating the three layers of the spore wall (exine). The innermost layer (brown) is thin (15 µm), the central, iridescent layer is thicker (20 µm), and the outer layer in this species (pale brown) forms the reticulate ornament on the wall and is thus up to 40 µm in thickness. Scale bar = 150 µm.

Figure 5. The iridescent central layer of the spore wall can be seen clearly in this abraded specimen of *Erlansonisporites* sp. Note the range of iridescent colours. Scale bar = 180 µm.

Figure 6. A highly abraded specimen (lacking outer layer) of *Erlansonisporites* sp. exhibiting considerable iridescence including the red and orange part of the spectrum. Scale bar = 180 µm.

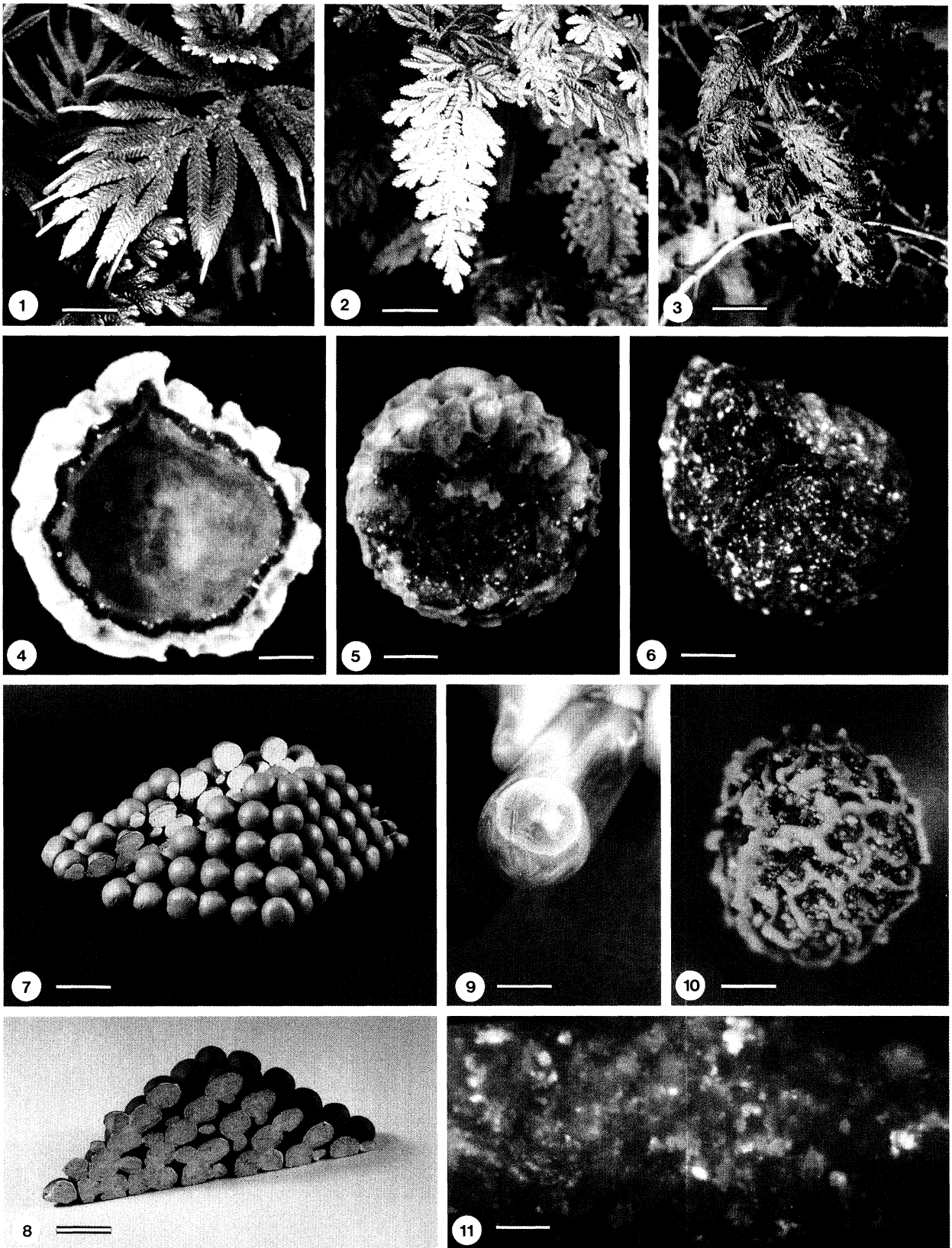
Figure 7. A model illustrating the close-packing system of the colloidal crystal forming the central layer of the exine. It is the regularity of the structure that gives rise to the iridescent properties of this layer (see figure 25). Scale bar = 20 mm.

Figure 8. The internal structure of the model illustrated in figure 7 shows the same features as are seen in TEM sections of the central exine layer (see figure 13) and in sections of other close-packed constructions. Scale bar = 20 mm.

Figure 9. Purified pellet of invertebrate iridescent virus type 22 (genus: *Iridovirus*). Iridescence here results from a close-packed structure (figures 15 and 16). Scale bar = 15 mm.

Figure 10. A different species of *Erlansonisporites* exhibiting a less ‘sharp’ form of iridescence. This results from rather less regularity in the arrangement of the constituent units of the spore wall. Scale bar = 180 µm.

Figure 11. This precious opal is a colloidal crystal preserved in a silica matrix. Its structure (essentially the similar particle size) makes it an excellent comparison with the spore wall colloidal crystal. As can be seen here, the range of colours resulting from the iridescence is similar to that in figures 4–6. Scale bar = 1 mm.



Figures 1 — 11. For description see opposite.

construction, the problem of how this high degree of order could have been formed 'biologically' still remained. The question of why such a structure should be produced and what biological function it may perform had also been raised, but no plausible answers had been forthcoming.

Encouraged by the earlier references to physical processes involved in the production of spore surface patterning, some of the current authors embarked upon an investigation of the mode of production of similar structures of non-biological origin. Following Taylor & Taylor's lead, opals (figure 11) were studied in more detail, but of particular interest was the realization that opals were colloidal crystals (regular sized spheres arranged and fixed in a silica matrix, illustrated by Darragh *et al.* 1976). Some of us (Hemsley *et al.* 1992; Collinson *et al.* 1993) have previously explored the relation between the opal structure and that of the spore walls discussed here. Similar structure (with the associated iridescence) has also been produced from colloidal polystyrene latex (Everett 1985; Pieranski 1983). Returning to biological systems, certain invertebrate viruses (Iridoviridae) also display iridescence due to their regular icosahedral structure and colloidal crystal arrangement in the cytoplasm of the host cells (Devauchelle *et al.* 1985; figures 9, 15 and 16). It had to be accepted that, as all of these systems of regular particles with iridescent properties were colloidal in origin, it was not unreasonable to consider the possibility that the spore walls were also colloidal in construction.

It is important to note that the leaf iridescence of some species of *Selaginella* results from a rather different arrangement, and that it is probably coincidental that iridescence occurs in both spores and leaves within the same genus. It should be remembered that spore iridescence is not generally visible until the outer layers of the spore wall (with different organization to the iridescent layer) are removed.

The aim of this paper is to offer a colloidal model of construction which does not conflict with the known ultrastructural development of *Selaginella* megaspore walls, and to explain the iridescence in terms of the underlying physics. It will be explained how iridescence is related to particle size and organization in this and other examples. The significance of self-assembly, such as that seen in the colloidal spore walls, will be discussed in terms of entropy and potential energy savings for the organism. This also leads to the conclusion that self-assembly procedures in organismal development offer the basis for a saltationary view of evolution.

2. ORDER AND ENTROPY

With the sudden popularization of 'chaos theory' (Gleick 1987) came the understanding that small perturbations can have massive effects on large-scale systems; the precise effect they may have, however, cannot be extrapolated from any realistic degree of understanding of the initial perturbation. In essence, this implied serious problems with the 'reductionist'

view, that understanding all of the parts of a system allows an understanding of the whole. From chaos theory came also the counter view, that ordered systems can result from apparently disordered constituents (known as 'anti chaos'), and this concept was applied to biological systems by Kauffman (1991). Such ordering cannot violate the second law of thermodynamics, and must therefore lead to an overall decrease in free energy and associated increase in entropy.

An important aspect of any dynamic system is the change in entropy associated with the processes taking place. Entropy is very often ignored in the consideration of biological processes, possibly unwisely. It is a measure of the degree of disorder in a particular system, and is important because it supplies a preferred 'direction' for processes occurring in a system which itself is not at equilibrium (processes will occur in such a way that equilibrium, i.e. maximum entropy, is attained). The second law of thermodynamics states that

'... the processes most likely to occur in an isolated system are those in which entropy either increases or remains constant.'

Schrödinger (1967) offered as a definition of life 'it feeds on negative entropy...', i.e. living systems progress by the transduction of more ordered forms of energy (e.g. sunlight or other organisms) to less ordered (high entropy) forms of energy (e.g. heat); essentially, they make use of sources which are not at maximum entropy (Penrose 1990). Entropy can be expressed by the equation

$$S = k \ln \mathcal{D}, \quad (1)$$

where k is the Boltzmann constant ($= 1.381 \times 10^{-23} \text{ J K}^{-1}$), \mathcal{D} is a measure of the 'disorder' in the system (related to the number of ways in which quanta of energy can be arranged within the system), and S is its entropy. It follows that an increase in order, e.g. self-organization, in a system is accompanied by a decrease in entropy in the immediate system but not in the environment as a whole where entropy must increase or remain constant. However, entropy can be reversed by an input of (low entropy) energy (Q),

$$\Delta Q/T = -\Delta S, \quad (2)$$

where T is the temperature of the system. Transduction of this energy by the organism will result in an increase in S (exceeding the local reversal) elsewhere. It can be seen, therefore, that organisms can create local order with an input of energy, but where ordered structures can be formed by a spontaneous local decrease in free energy (increase in entropy) of the components, then these represent a potential reduction in the energy expenditure of the organism.

Normally, therefore, changes in a system which result in greater order are driven by a decrease in potential (free) energy of the system, through attractive interactions between the constituents. However, in dispersions of hard-sphere particles (such as the exine components discussed here where

such attractive forces are absent), an order-to-disorder phase transition is known to occur at sufficiently high particle volume fractions, which is entropy driven: the particles then rearrange themselves such that there is a net increase in entropy (Alder & Wainwright, 1962). This point is discussed in detail below. The particles forming the regular exine layer in the megaspore wall clearly reach a sufficiently high particle volume fraction to be subject to such effects.

3. THE COLLOIDAL SYSTEM OF EXINE CONSTRUCTION: AN EXAMPLE OF SELF-ORDERING

As briefly discussed above, the exine of the particular species of *Erlansonisporites* (fossil *Selaginella* megaspore) in question consists of three layers, each with a different ultrastructure. The inner layer consists of particles of an irregular shape, but each with the same thickness, i.e. they may be spheres, rods or discs of exine material. The central (iridescent) layer (figure 4) is composed of spherical (or nearly spherical) particles which are arranged in a highly ordered way and which form compound units separated by dislocations: the colloidal crystal (figure 13). Lastly, the outer layer (which supports and is continuous with the surface ornament) consists of laminae which are highly convoluted. These three layers are described in detail by Hemsley *et al.* (1992) and Collinson *et al.* (1993). An important feature of these layers is that the interface between each is not abrupt but gradual, implying that the same process of exine deposition, or at least variations on this, occurred over the entire period of formation, i.e. it is unlikely that a colloidal mode of exine development is restricted to the

iridescent central layer. If the colloidal model is to be acceptable it must account not just for the regular layer of the exine, but for the adjacent, less regular layers.

It is not wholly clear how the particles, of which the exine is composed, are produced. Studies of *Selaginella galeottii* Spring (Taylor 1991*b*) demonstrate that particles appear suddenly and that they appear at a much smaller diameter (0.035 μm as opposed to 0.24 μm in mature spore walls). The expansion of the particles seems likely to occur by way of accretion of additional material (sporopollenin) from the sporangial contents surrounding the particles. This presumably occurs at a steady rate on all surfaces until the concentration of the sporopollenin precursor drops to a low level.

'Seeding' of exine particles followed by rapid growth seems a likely explanation for development.

We must now turn to an explanation of how particles 'aggregate' to form the structures we observe in the three layers and the transitions between these layers.

Figure 21 shows a schematic 'phase map' for a dispersion of weakly attracting particles. For dispersions of hard-sphere particles (possessing no attractive forces), between particle volume fractions of 50% and 55%, it has been predicted theoretically (Alder & Wainwright 1962) that the dispersion spontaneously separates into two coexisting colloidal phases: a disordered phase (volume fraction 50%) and an ordered, crystalline phase (volume fraction 55%). This phase change is entropy driven, i.e. there is a net gain in overall entropy. Below 50% volume fraction only the disordered phase exists; above 55% (up to 74%, when the particles close-pack) only the crystalline phase exists. Direct experimental evidence

Figure 12. An SEM image of the exine of *Selaginella galeottii*. The inner layer consists of irregular particles whereas the central layer can be seen to consist of regularly arranged particles, packed in an orderly way. This megaspore wall differs from that illustrated in figure 4 in that the outer layer is thin and the central layer participates in the formation of the outer reticulum. Scale bar = 10 μm .

Figure 13. A TEM image of the constituent particles of the central layer of *Erlansonisporites* sp. Their organization into domains separated by dislocations is evident (see also figure 14). Scale bar = 0.75 μm .

Figure 14. A TEM image of a section through much of the wall of *Erlansonisporites* sp., illustrating particularly the inner and outer boundaries of the colloidal crystal layer. Note the very different appearance of the outer exine layer (top), as compared with the other layers, and a superficial resemblance to a periodic minimal surface. Scale bar = 4 μm .

Figure 15. Crystalline arrays of *Iridovirus* type 22 particles in host insect tissues (*Simulium* sp.). Note particularly the similarity of appearance of this section with that of the spore wall (figure 14). Scale bar = 2 μm .

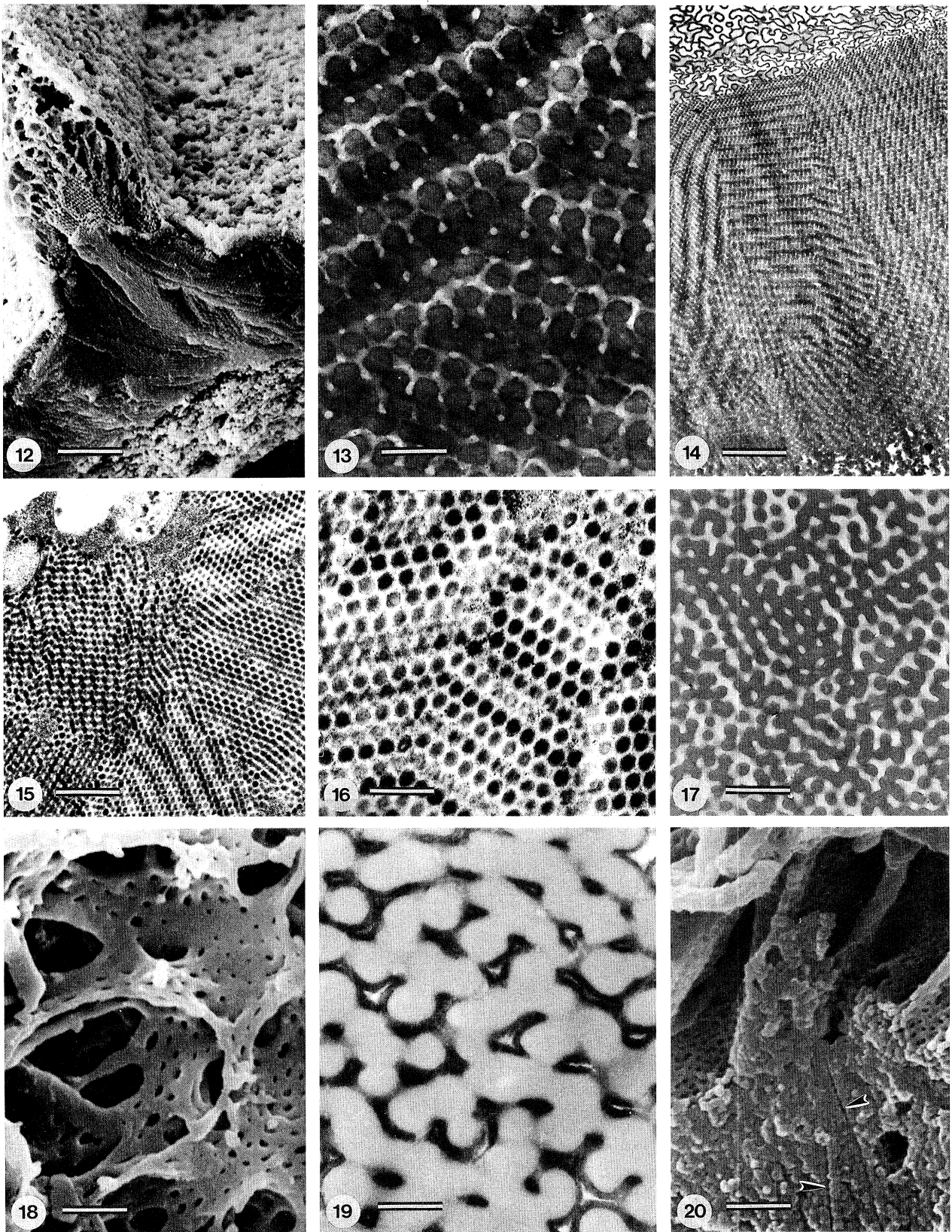
Figure 16. Detail of the regular structure shown in figure 15, and illustrating similar features to figure 13. Scale bar = 600 nm.

Figure 17. TEM image of *Selaginella*-like megaspore wall with consistent particles of more or less similar size, but less regular arrangement than in the previously illustrated iridescent species. Scale bar = 1.5 μm .

Figure 18. SEM of surface of *Selaginella*-like megaspore. Note the variable scale of ornament and cavities. Scale bar = 1.2 μm .

Figure 19. Detailed TEM image of an *Erlansonisporites* spore similar to that illustrated in figure 10. The constituent particles are of more variable size and less regular arrangement than in the previously illustrated iridescent species. This results in a poorer quality iridescence without the speckled effect obtained from the colloidal crystal. Scale bar = 0.23 μm .

Figure 20. SEM image of *Selaginella*-like megaspore wall showing transition between more or less regular and outer irregular zones. Note channels (arrowed) which may represent the original position of the long-chain polymers involved in the process of depletion aggregation. Scale bar = 2 μm .



Figures 12–20. For description see opposite.

for this behaviour with hard-sphere dispersions has been obtained by Pusey & van Megen (1986).

If a weak attractive force is superimposed then, as illustrated in figure 21, the two-phase coexistence region 'widens'. Such an attractive force can be present if there is a significant concentration of

polymeric material in solution. This situation is illustrated in figure 22. When two particles approach to a separation less than the dimensions of the polymer molecule in solution then, for entropic reasons, the polymer tends not to enter the gap between the particles (to do so would mean that the

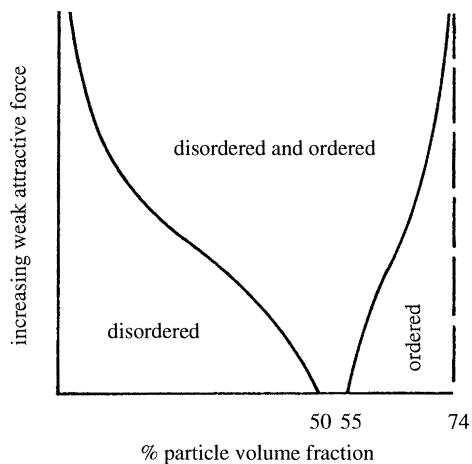


Figure 21. A plot of particle volume fraction (as a percentage) against strength of attractive force between particles. The horizontal axis ranges from 0% to 74%, as this is the maximum volume occupied by close-packed particles. The vertical scale depicts the effect of an attractive force, initially at zero. The baseline therefore shows the behaviour of particles that adhere on contact but do not exhibit any attraction. Different conditions (attractive or repulsive forces) would give other shapes of curve. The phase of coexisting ordered and disordered particles would be much like that illustrated in figure 17. This phenomenon is described in detail by Vincent (1987), from which this diagram is redrawn.

polymer would be 'constrained', i.e. it could not adopt as many configurations as in the 'free', solution state). As a result, the gap between the particles is effectively devoid of polymer, thus an osmotic pressure gradient exists between this region and the bulk polymer solution. Consequently, solvent will spontaneously move out of the gap region into bulk, thus drawing the particles together. This effect is known as 'depletion attraction' (Napper 1983). Coalescence of the colloidal particles gives rise to structure showing

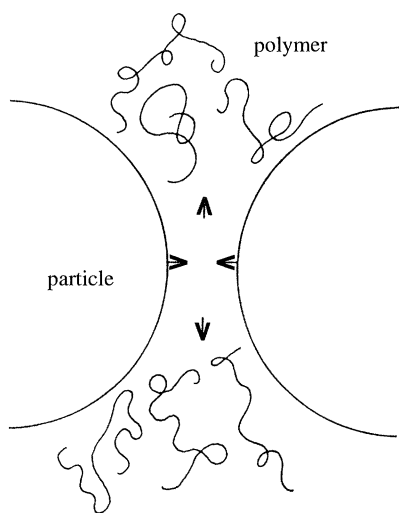


Figure 22. Depletion attraction requires the presence of a long-chain polymer amongst the particles which are to aggregate. As the particles approach each other, the polymer (due to its size) is excluded from between them. Osmosis then serves to draw the supporting fluid from the space, thus drawing the particles even closer together.

the features of a periodic minimal surface (Yoshimura & Hachisu 1983; Thomas *et al.* 1990), e.g. figure 14.

We may now postulate the following model for the three-layered structure observed in the megaspore walls of *Selaginella* and *Erlansonisporites*. Initially, the spore wall consists of a mixture of colloidal sporopollenin particles and some (as yet undefined) polymeric material dissolved in solution. Over time, water is withdrawn from the spore walls, leading to a build up of polymer concentration. At some critical polymer concentration, colloidal phase-separation starts to occur commencing near the outside of the wall. This leads to two coexisting colloidal phases in this region: one rich in particles, which forms the (central) crystalline region, the other dilute in particles. Further withdrawal of solvent (water) from this dilute (outer) region leads to slow flocculation of these particles into the sheet-like structures depicted in figures 12, 14, 18 and 20. The 'inner' layer (concentrated in particles, but not crystalline), would result, if water loss were to be suppressed sufficiently, such that the polymer region does not exceed the critical concentration for colloidal phase-separation.

Evidence supporting this model of formation was limited to the close similarity of structures such as those shown in figures 18 and 20 to flocculation of colloids produced by depletion attraction. However, Morbeli & Rowley (1993) have reported 'wicks' (their term) seen in developing *Selaginella* spore walls. These wicks consist of numerous fibre-like structures among the developing exine components. Such wicks have also been observed by W. A. Taylor (personal communication). In our view it is highly likely that these fibres are formed from the long-chain polymers

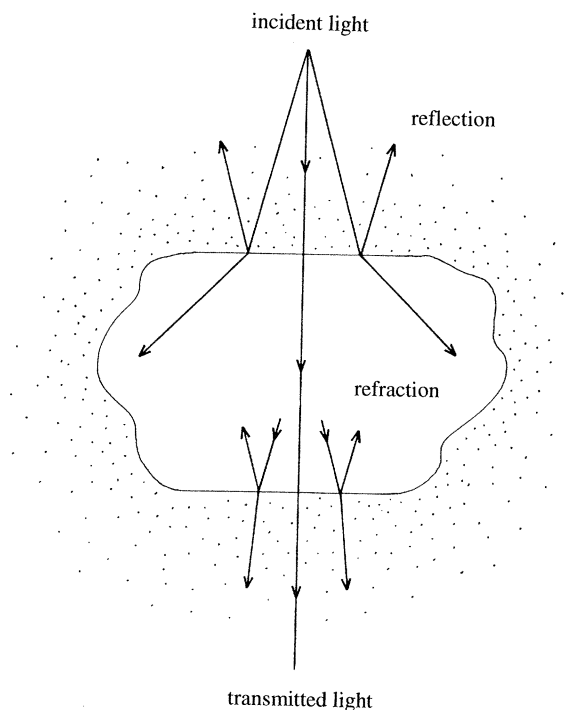


Figure 23. As light passes from one medium to another, it may be scattered by reflection or refraction. In spore walls, cavities (air or fluid filled) may serve to cause considerable scattering.

responsible for the depletion attraction assembly. The channels in figure 20 (arrowed) may represent the original positions of these long-chain polymers.

4. IRIDESCENCE AND LIGHT SCATTERING IN FILMS AND COLLOIDAL CRYSTALS

The ultrastructure of a spore exine, particularly that of a megaspore, affects its appearance, especially the transparency of the exine and to some extent its colour. These features are closely linked to the frequency of occurrence of cavities or different layers within the wall. Cavities are important because they provide junctions between solid sporopollenin (which appears to be more or less transparent) and air–water. It is at these junctions that light refraction and reflection can occur (figure 23). The greater the number of cavities (and hence junctions), the more opaque the spore wall. This is equivalent to considering the relative transparency of a sheet of glass compared with an equally thick sheet of glass fibre. It is important to note that the scattering (loss of transparency) is reduced if the refractive index

between cavity content and exine material is reduced (e.g. glass slides are less visible in water).

Spongy spore exines (figure 24, left) may consist of less material than more solid walls (figure 24, right) and yet be more opaque. This is the case with a number of fossil spores belonging to the genus *Lagenicula*. The exines of *Selaginella* are of the spongy type with many cavities, as are the fossil species of *Erlansonisporites* (clearly seen in figure 12). Considerable light scattering occurs, making the spore exine opaque. However, it is the spongy nature of the spore wall, or at least its particulate nature, that gives rise to the related optical phenomenon of iridescence.

Iridescence in the leaves of *Selaginella* results from the interference of white light reflected from a thin film of material at the leaf surface (see Héban & Lee 1984; Lee 1986). This reflection is made possible by the junction between the thin film and its different surroundings. Film thickness (d) and colour (λ) are related by the equation

$$2 \mu d \cos \theta + (\lambda/2) = m\lambda, \quad (3)$$

where μ is the refractive index of the film, θ is the angle of observation relative to the normal, and m is

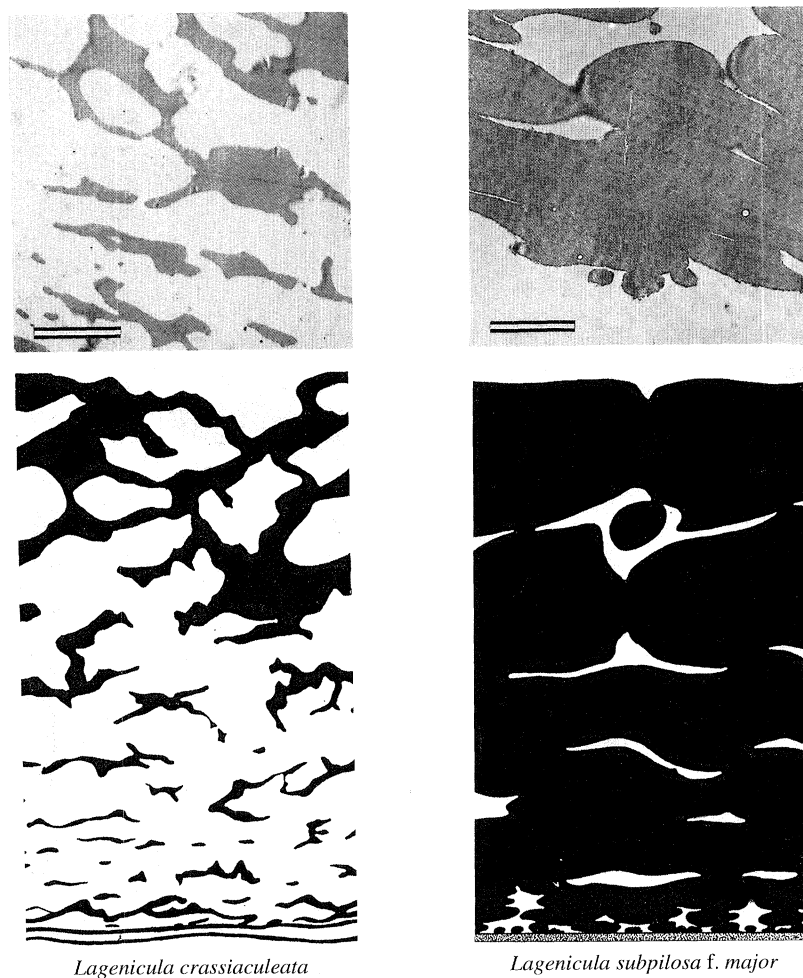


Figure 24. Examples of two spore wall types (from related Carboniferous species). On the left, a low density exine which is thoroughly opaque due to light scattering at the numerous interfaces between the wall material (black) and the cavities (white). On the right, a wall of similar thickness but in which there are fewer cavities, with the result that the spore appears more translucent. Upper illustrations are TEM sections of the outer portion of the megaspore wall, lower illustrations are diagrammatic illustrations of the entire wall thickness. Scale bar left = 3 μ m, right = 1.2 μ m.

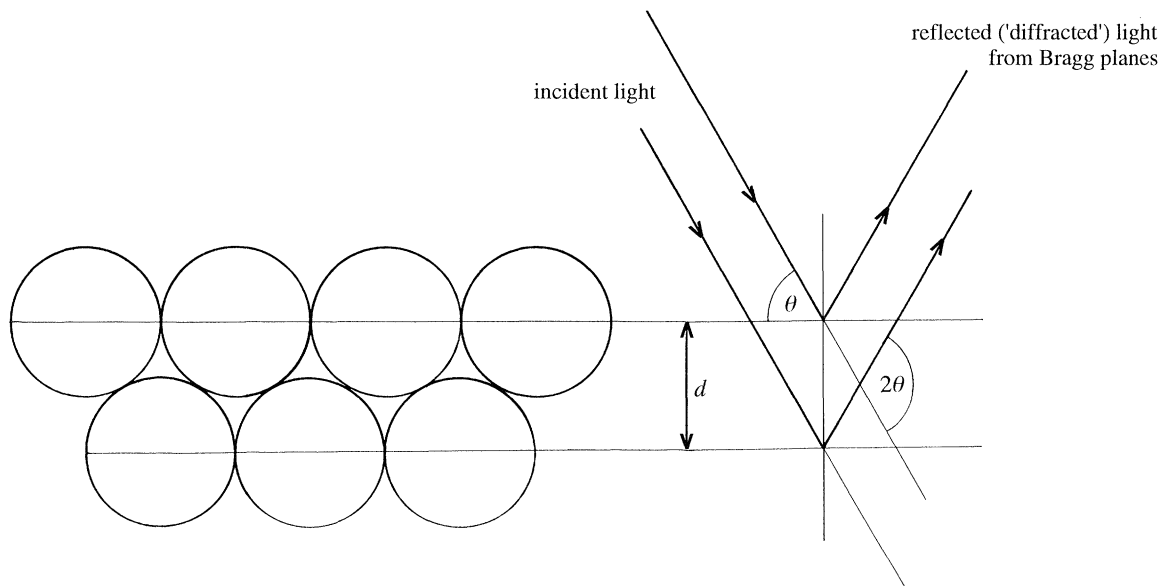


Figure 25. The arrangement of the particles in the colloidal crystal gives rise to a series of Bragg planes (two of which are shown in an approximation of their arrangement in the spore wall). Reflection from these planes results in constructive superposition of light under particular circumstances.

an integer. If m is taken as 1 and θ is 0° ($\cos \theta = 1$) then the equation can be written in the form

$$2 \mu d + (\lambda/2) = \lambda, \quad (4)$$

or

$$4 \mu d = \lambda. \quad (5)$$

Hébant & Lee (1984) provided examples of how this was used to estimate the film thickness before it was measured in TEM section.

The iridescence found in the spore walls results from reflections from the multiple layers of regularly arranged particles (figure 25) as opposed to a single layer. Due to the consistency of particle diameter, the layers (Bragg planes) within the colloidal crystal are also of regular spacing. This results in constructive superposition governed by the Bragg equation:

$$m\lambda = 2 \mu d \sin \theta. \quad (6)$$

In this equation, θ is the angle of observation relative to the plane, as opposed to the previous example where θ was measured relative to the normal; λ is the refractive index of the sporopollenin. To equate the two situations we may take θ to be 90° such that $\sin \theta$ becomes 1. Again taking m as 1, the equation to find the maximum observable wavelength of constructive superposition (λ) becomes

$$\lambda = 2 \mu d. \quad (7)$$

This equation relates the distance between the layers in the crystal (d) to the iridescent wavelength (colour). In a close-packed, tetrahedral arrangement of spheres, the distance between the Bragg planes is related to the particle centre-to-centre spacing (the same as particle diameter and denoted by D , figure 26) by

$$d = \sqrt{\{D^2 - [(D/2) \cos 30^\circ]^2\}}. \quad (8)$$

Substituting this into (7) we obtain

$$\lambda = 2 \mu \sqrt{\{D^2 - [(D/2) \cos 30^\circ]^2\}}, \quad (9)$$

which provides a direct relation between particle size and iridescent colour. This is applicable to any iridescent regular close-packed system, and so may be used for iridescent virus aggregates as well as spore walls. The only unknown in this equation is the refractive index (μ). For the spore wall, taking μ as 1.52 (based on an average value for synthetic polymers listed by Hemsley (1984)) and mean spore wall particle diameter as $0.24 \mu\text{m}$ (see Hemsley *et al.* 1992), we obtain a value of 657.6 nm for λ which corresponds to a red colour.

Invertebrate iridescent viruses are classified according to particle size: the larger *Chloriridovirus*

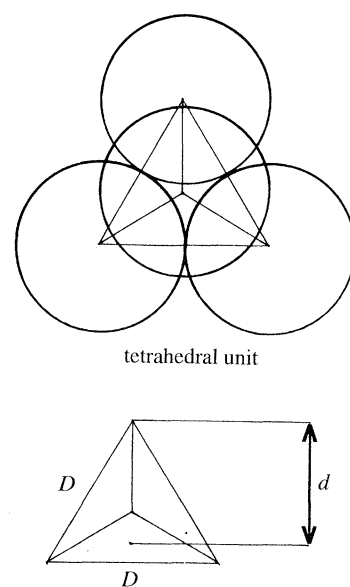


Figure 26. In a close-packed system of spherical particles, the distance between the Bragg planes (d) is related to the centre to centre spacing (D), also equivalent to the sphere diameter; d (in the three-dimensional system) is therefore slightly smaller than in the diagrammatic example in figure 25.

genus (around $0.18\ \mu\text{m}$) and the smaller *Iridovirus* genus (around $0.13\ \mu\text{m}$) (sizes derived from sectioned EM material). However, when particles are negatively strained, a technique causing less shrinkage of specimens, values of $0.224\ \mu\text{m}$ and $0.17\ \mu\text{m}$ have been obtained for typical chlorido- and irido-viruses, respectively (Wagner *et al.* 1973; DeBlois *et al.* 1978). By using these values for D in (9), we obtain predicted iridescence wavelengths of $613\ \text{nm}$ (orange) for chloriridoviruses and $465\ \text{nm}$ (blue) for iridoviruses, which are indeed the colours observed.

Some spores produce a poor iridescence with clearly impure colours (figure 10). This results from a slightly more irregular arrangement of particles and variable particle size. These two factors clearly have a direct bearing on d (see figure 26 and equation (8)) and result in constructive superposition over a range of wavelengths. This effectively means that the flecks of iridescence are no longer monochromatic, nor is the angle of observation so critical. Iridescence is therefore not confined (in the case of spore walls) to the close-packed example, but may also occur in systems possessing lower particle volume fractions (in the 50–55% range in figure 21). Examples of this type of exine organization are illustrated by Collinson *et al.* (1993) and figures 17 and 19.

5. SELF-ORDERING SYSTEMS IN DEVELOPMENT AND THEIR EVOLUTIONARY CONSEQUENCES

Consideration of self-ordering systems in biology has been largely overlooked. Complex membrane organization generally results from microtubules (which may themselves be self-assembling to a high degree), but the way in which the constituents of systems interact is poorly understood. It is important to realize, however, that a system which is self-ordering could well be incorporated into biological development if it represents a potential saving in terms of the energy input required for construction. Self-assembly also represents a much simpler (more parsimonious) evolutionary pathway; only a few genes that control the physical make-up of the solution are needed, in contrast to the very large number that would probably be needed to control an assembly mechanism for structures this complex and varied. Thus the actual appearance of the spore wall and its modification through time is a much simpler evolutionary process and therefore more likely to occur. Certainly, in the spore wall example described in detail above, formation as a self-assembling colloidal structure may require less energy input from the plant than alternative methods of exine construction.

Self-assembly on a smaller scale occurs in two dimensions in the construction of algal and bacterial membranes (Glauert & Thornley 1973; Roberts 1974), and certainly plays a part in the formation of tobacco mosaic virus particles, proteins aggregating around the RNA base (template) as a result of an associated change (reduction) of the free energy of the system (Caspar 1964). Icosahedral viruses result from attainment of a minimum free energy state amongst

the constituent wall units, and are in turn analogous to the many ‘unlikely’ chemical structures (supramolecules; see, for example, Seel & Vögtle 1992) which self-assemble around suitable templates. Each of these examples illustrates self-assembly at a different scale, spore walls being even larger in scale than virus aggregates, but in all cases organization results simply from the presence of the initial components and the thermodynamics of the system.

Self-assembly and the attainment of minimum free energy (maximum entropy) are closely related, as has been seen in relation to the colloidal systems discussed here. The main problem currently faced in determining the extent of self-assembly in biological systems is that, as in the above examples, the mode of formation is only revealed when there is regularity of the resulting structure. However, not all self-assembly systems need necessarily give rise to regular structure, as has been shown in the depletion attraction model presented here. It is probable, on thermodynamic grounds, that similar, non-regular self-assembly systems are widespread in biological development.

One good example of irregular self-assembly is that of the configuration of proteins. It is well known that proteins ‘roll up’ in a specific and well-defined way, a feature crucial to the implementation of their correct function. Disulphide bridges play a role in maintaining the resulting structure but are unlikely to be the cause of such a precise configuration (Dyson 1978). Clearly, one incorrect amino acid in the chain will potentially alter the final configuration, possibly enormously, thus rendering that protein non-functional (for its original purpose).

The suggestion that many biological constructions may be self-assembling is not a denial of the crucial role of the gene in determining the ultimate structure. What is suggested is that less information need be present on the gene to produce an equivalent structure, if self-assembly is involved (figure 27). In view of this, one might expect development of biological structures to consist of periods during which the conditions necessary for self-assembly are attained (direct genetic influence) followed by periods of self-assembly (independent of genetic influence). Kauffman (1991) has taken this concept further in suggesting that the form taken by many similar biological structures results more from organized systems attaining a (convergent) minimum free energy state than from the effects of natural selection. This view does provide an explanation of why and how colloidal systems of organization may have arisen in selaginellalean lycopsids. Possibly Kauffman (1991) is correct in that we witness a trade-off between the natural selection of form and the structure dictated by thermodynamics.

Clearly, development sequences which incorporate periods of self-assembly may well show a discontinuous range of variation in structure, especially if the self-assembly proceeds in a very different way with only very slight changes in the initial conditions. If such self-assembly systems are incorporated at critical stages in the development of an organism, small changes may

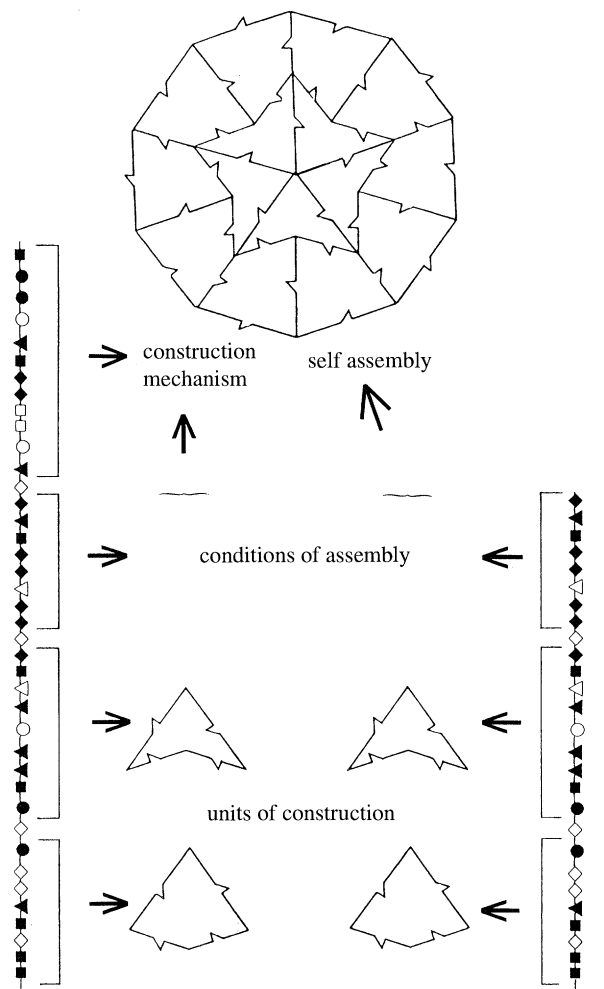


Figure 27. Systems in which self-assembly is used require fewer instructions for construction. Here, a form of information storage (the genes in a biological system) codes for the production of the basic units, and for the environmental conditions of construction (both left and right). Where self-assembly occurs (right), no further information input is necessary for the creation of quite complex structures, otherwise (left) additional information is required to code for the construction mechanism. 'Units of construction' after Penrose (1990).

result in apparently massive alteration of the final structure. Such a system may be viewed as offering a pathway for sudden, significant changes in biological molecular structures, which may in turn be seen as leading to saltation in the evolutionary process.

6. CONCLUSIONS

The colloidal system discussed here has led to some important concepts regarding the formation of structures during exine development. Depletion attraction provides a mechanism for the self-assembly of the entire exine, both of the regular central layer and of the surrounding irregular layers. The special conditions present during development of the central layer give rise to iridescence, but apparently no striking features characterize the forms taken by the colloidal sporopollenin in the irregular layers (and in this they resemble the exine of most other lycopsid

megaspores). The most important aspect of this model is that it indicates that non-regular self-assembly leads to differences in organization of the zones in spore walls. The model also suggests that irregular self-assembly plays a role in biological development, and may result in major differences in the appearance of organisms with a very similar genetic configuration.

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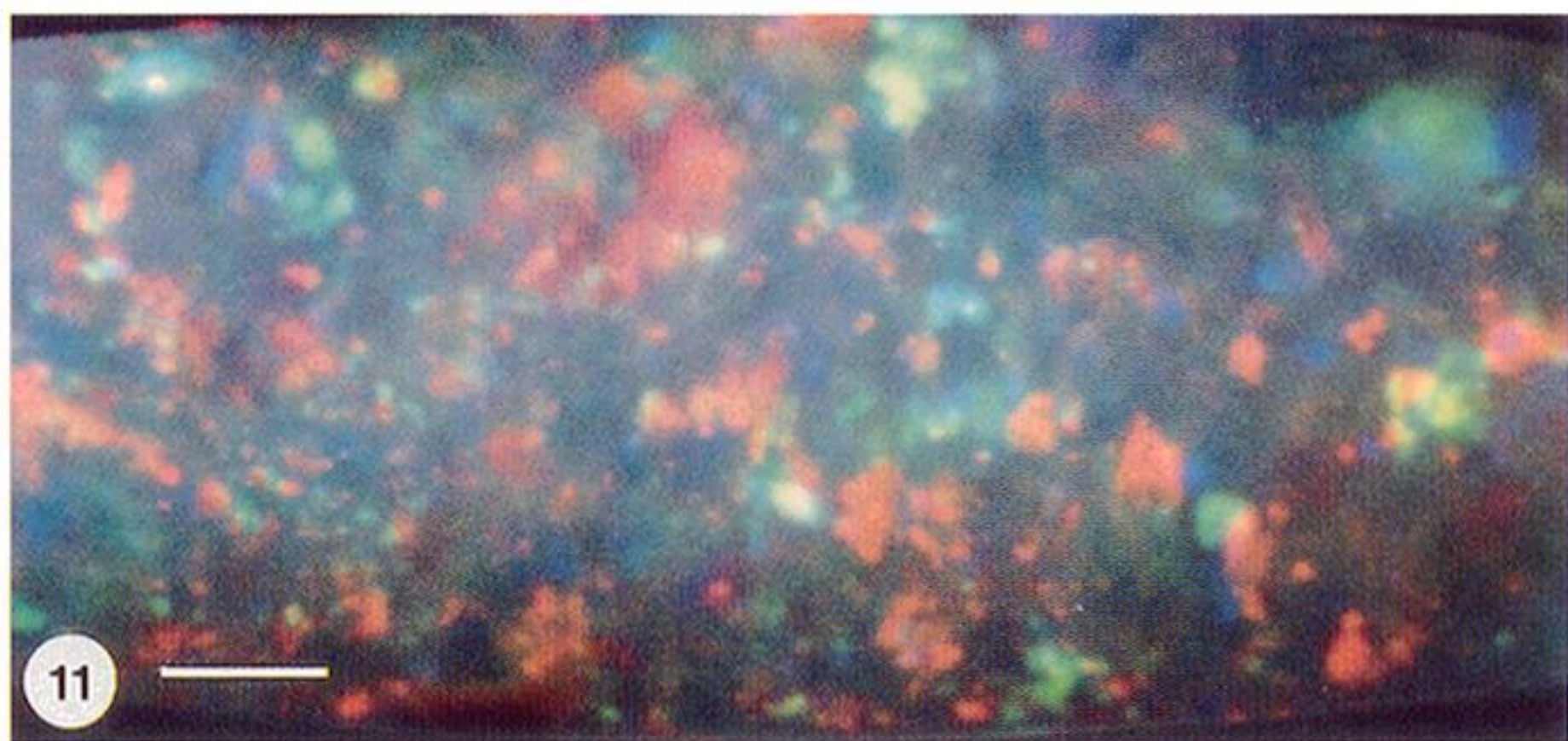
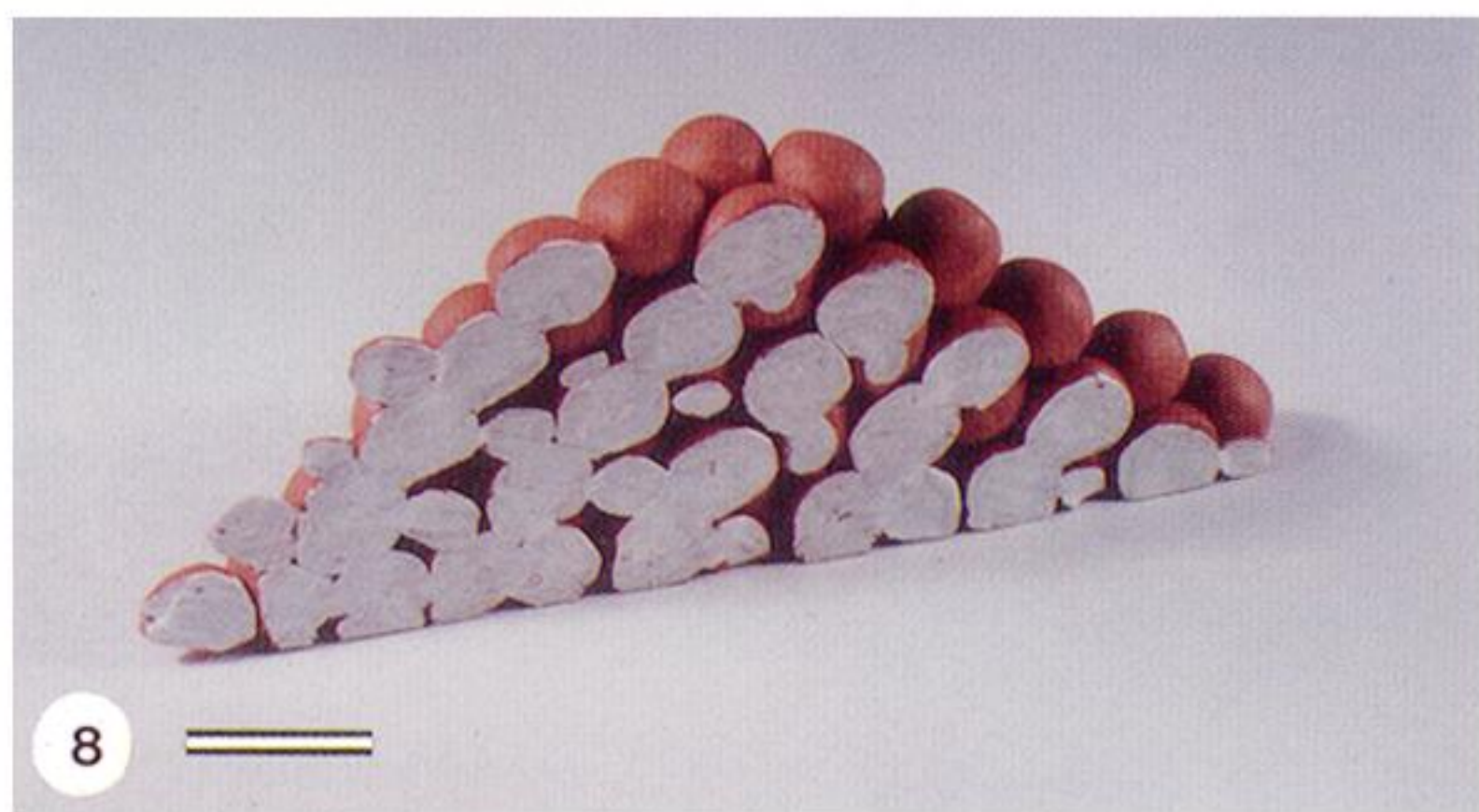
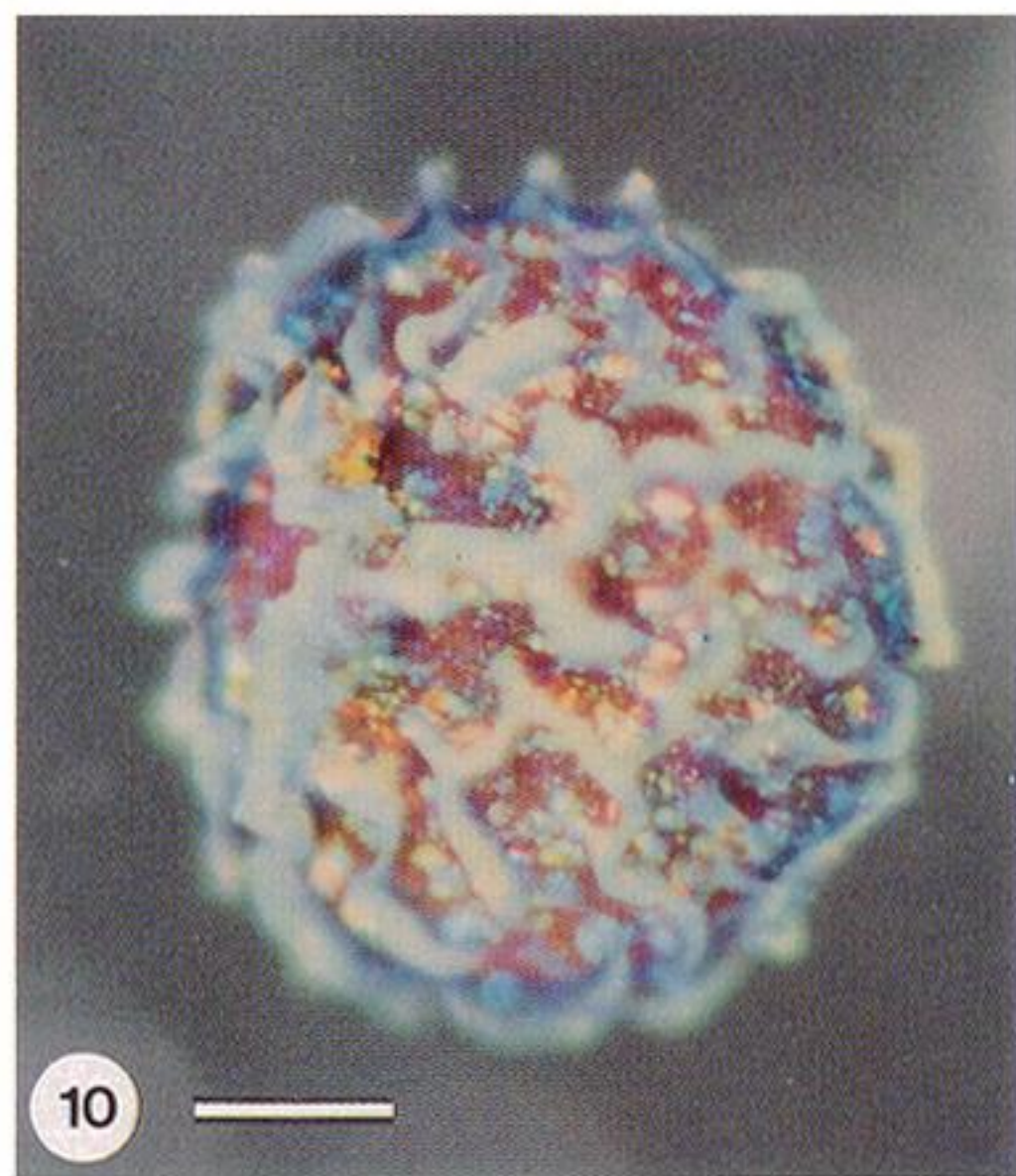
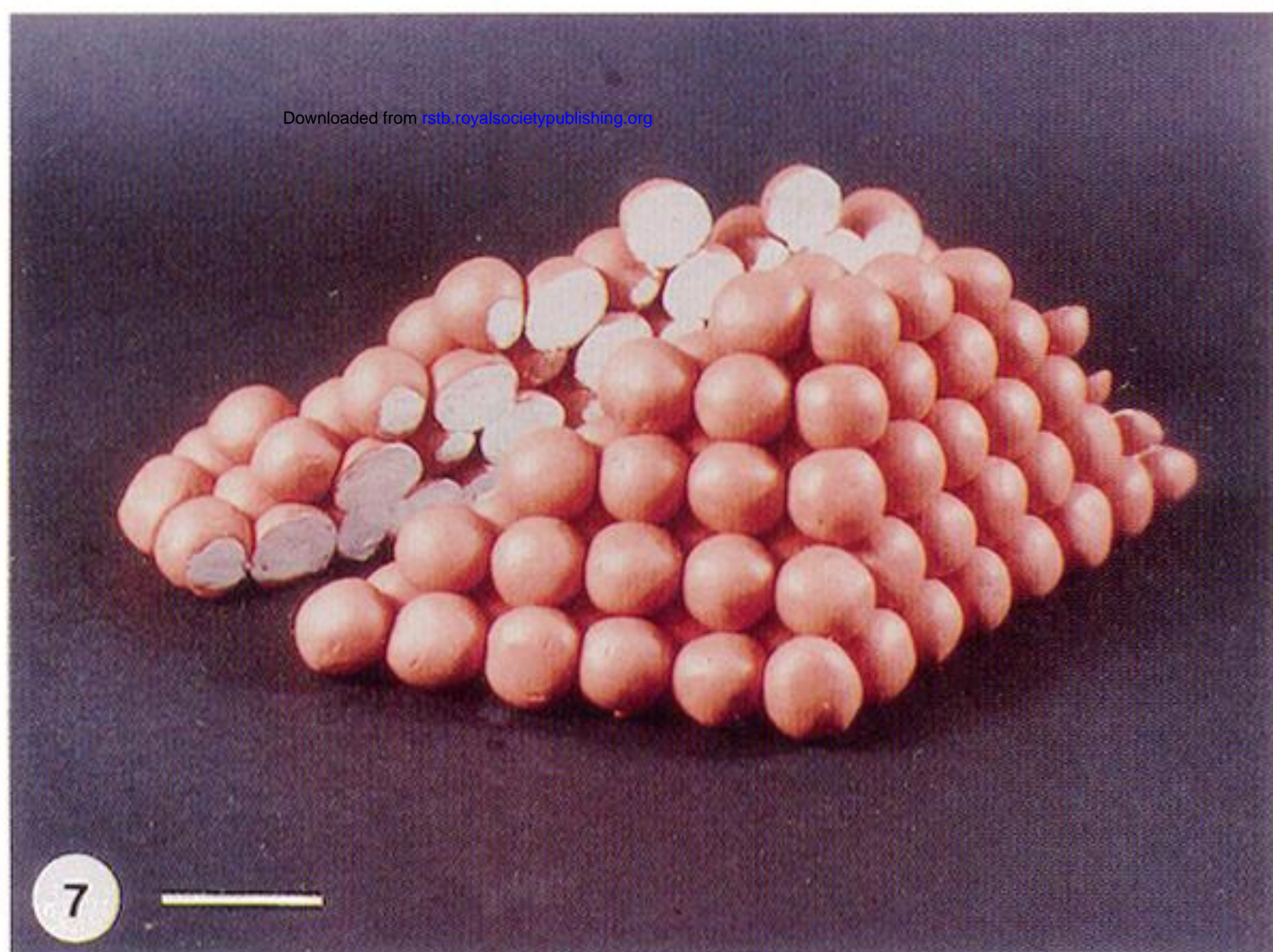
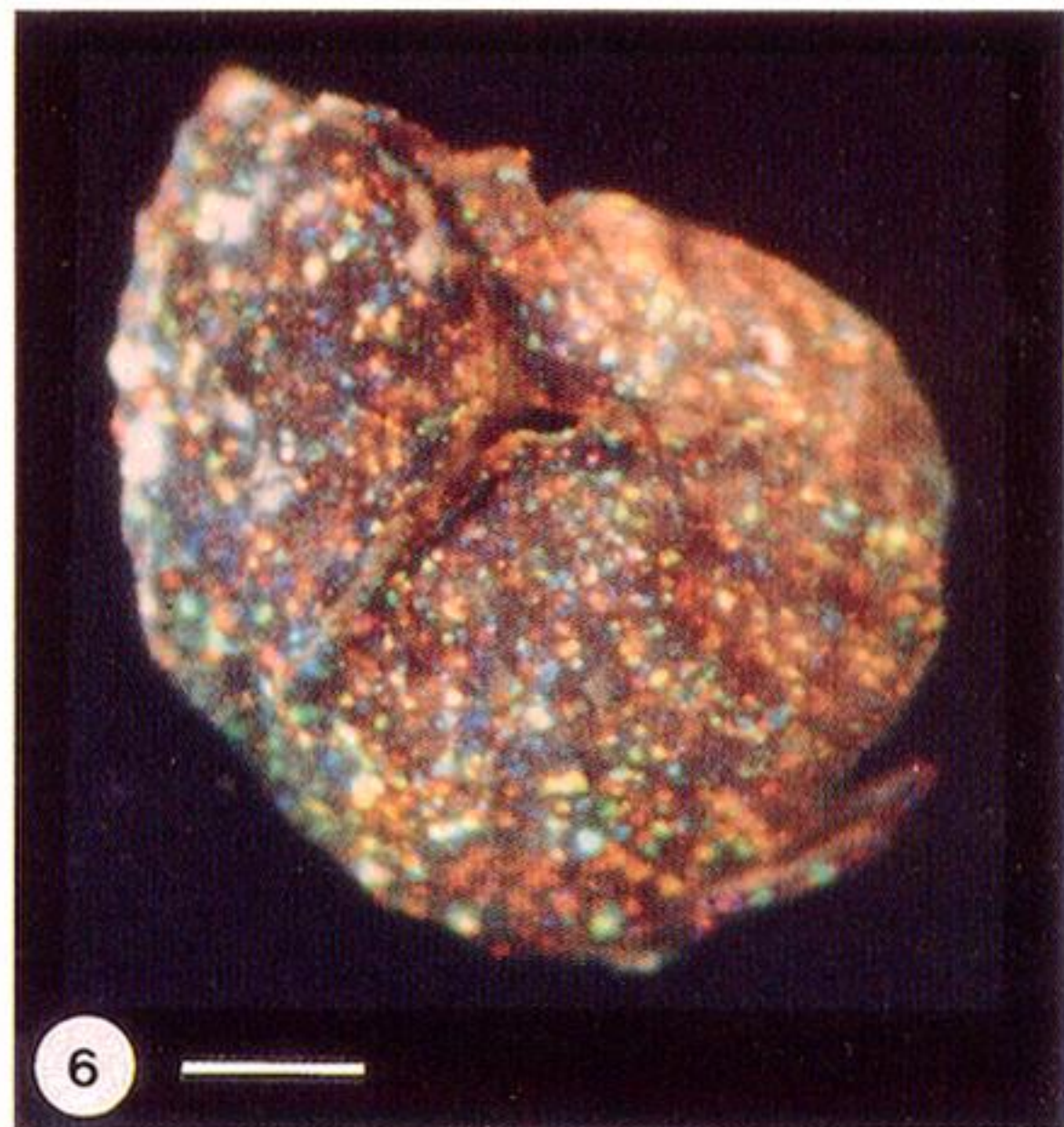
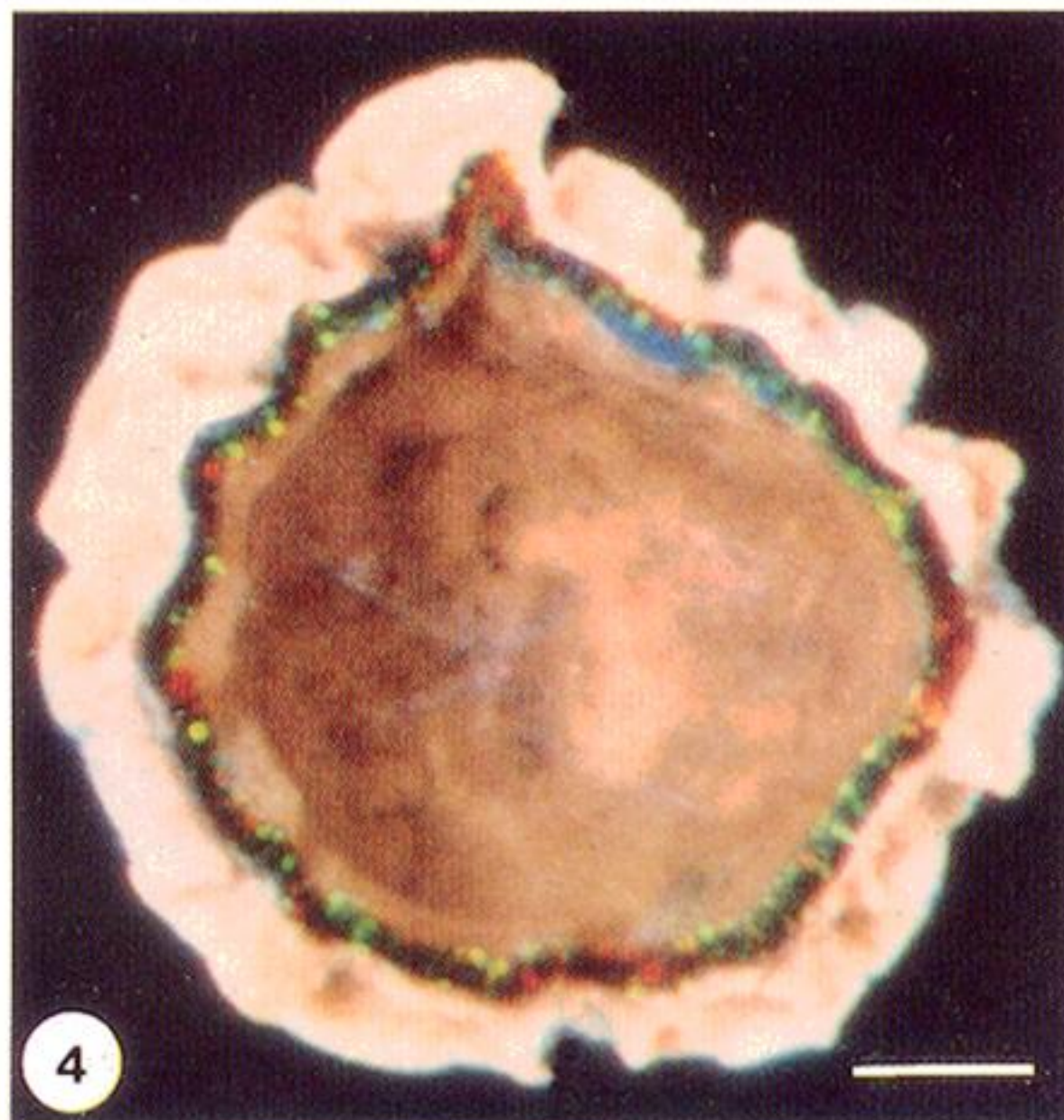
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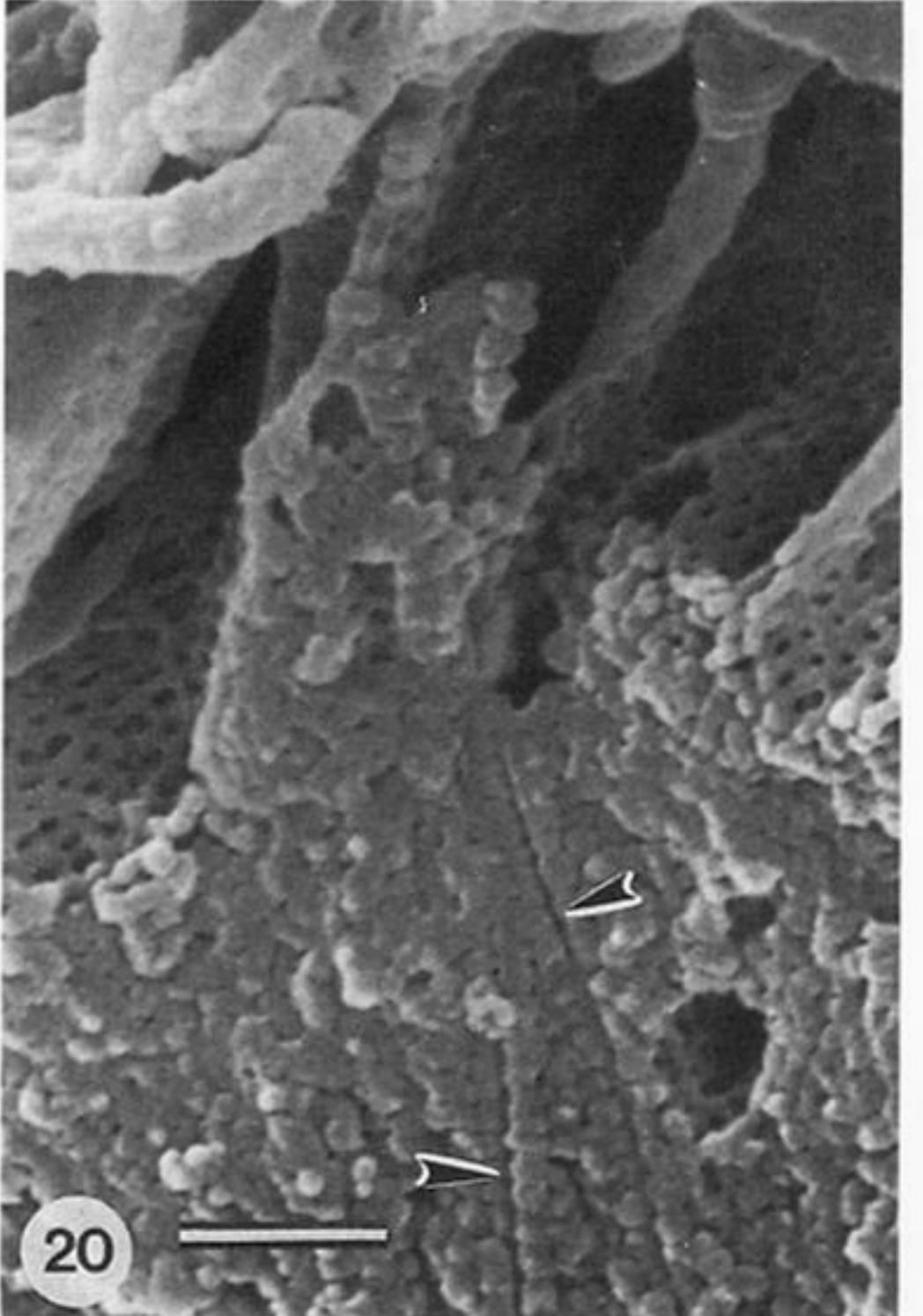
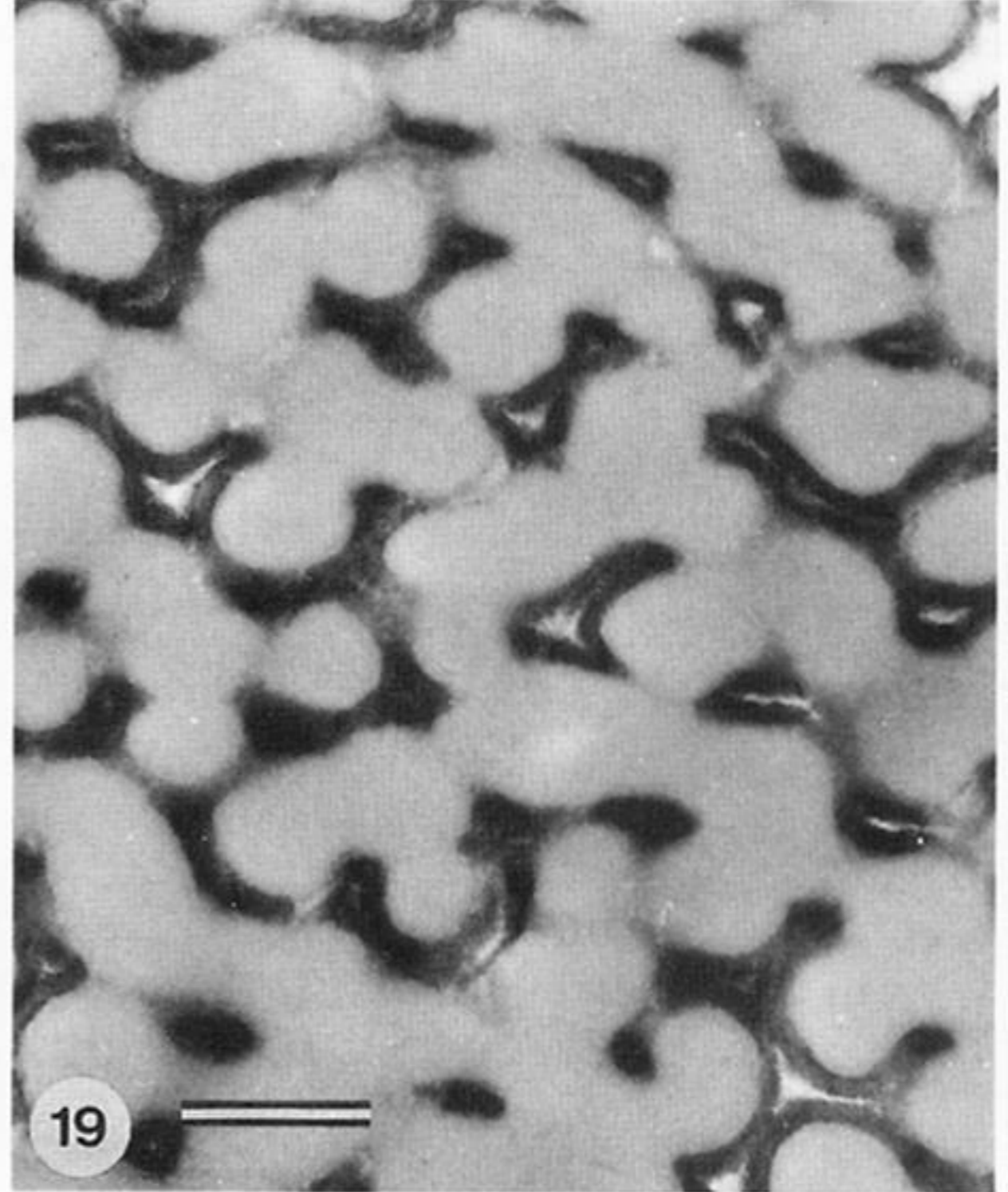
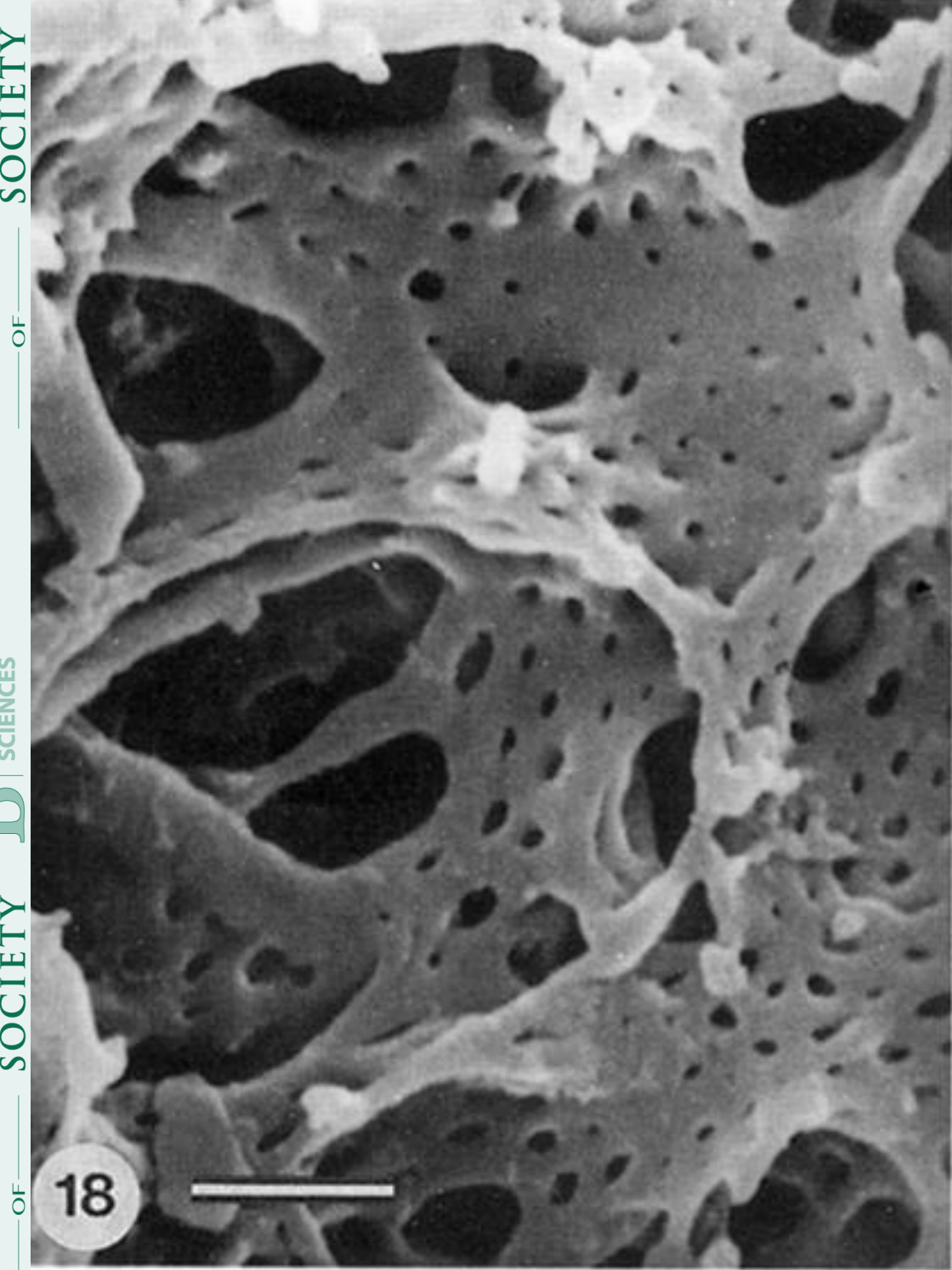
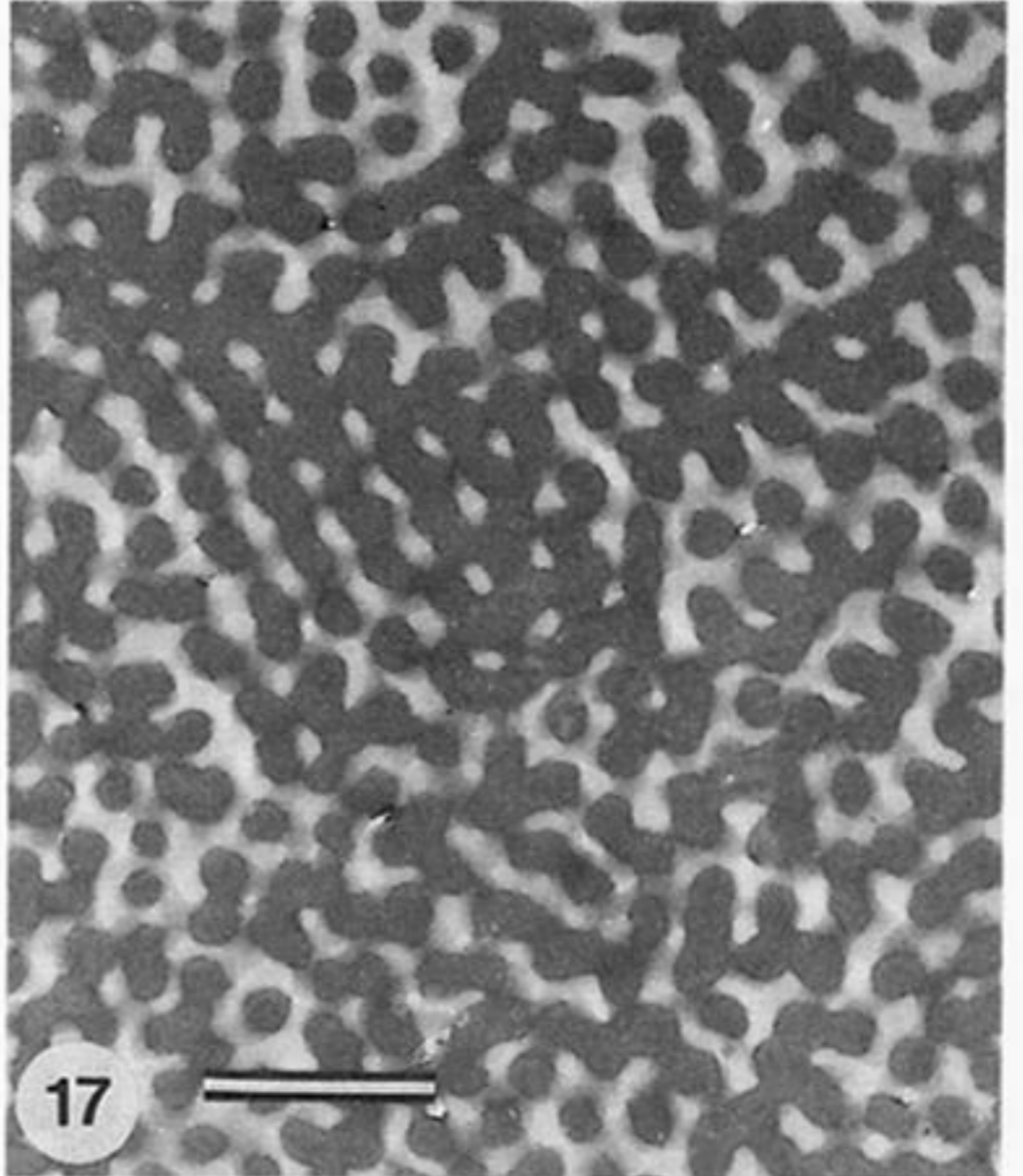
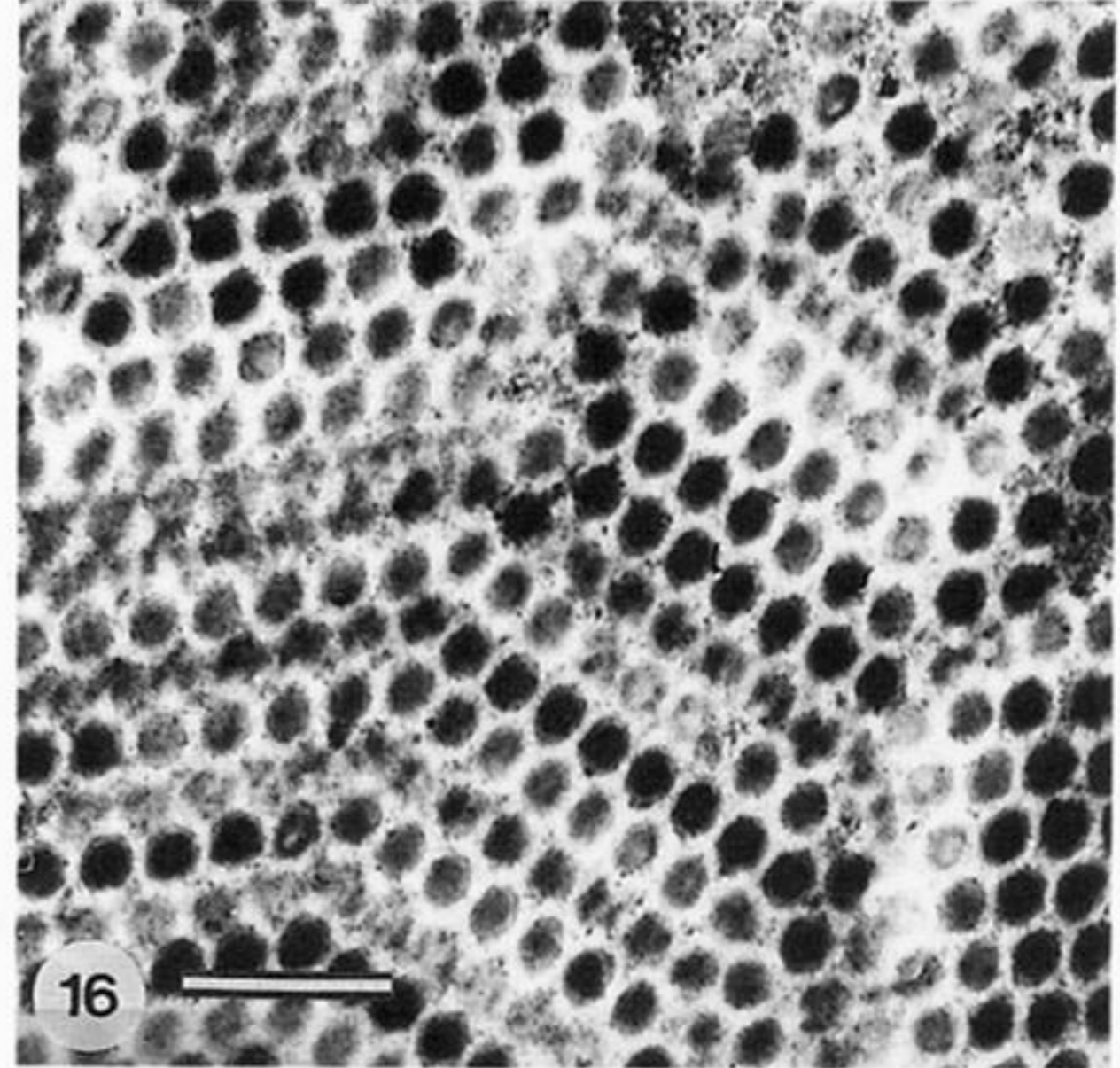
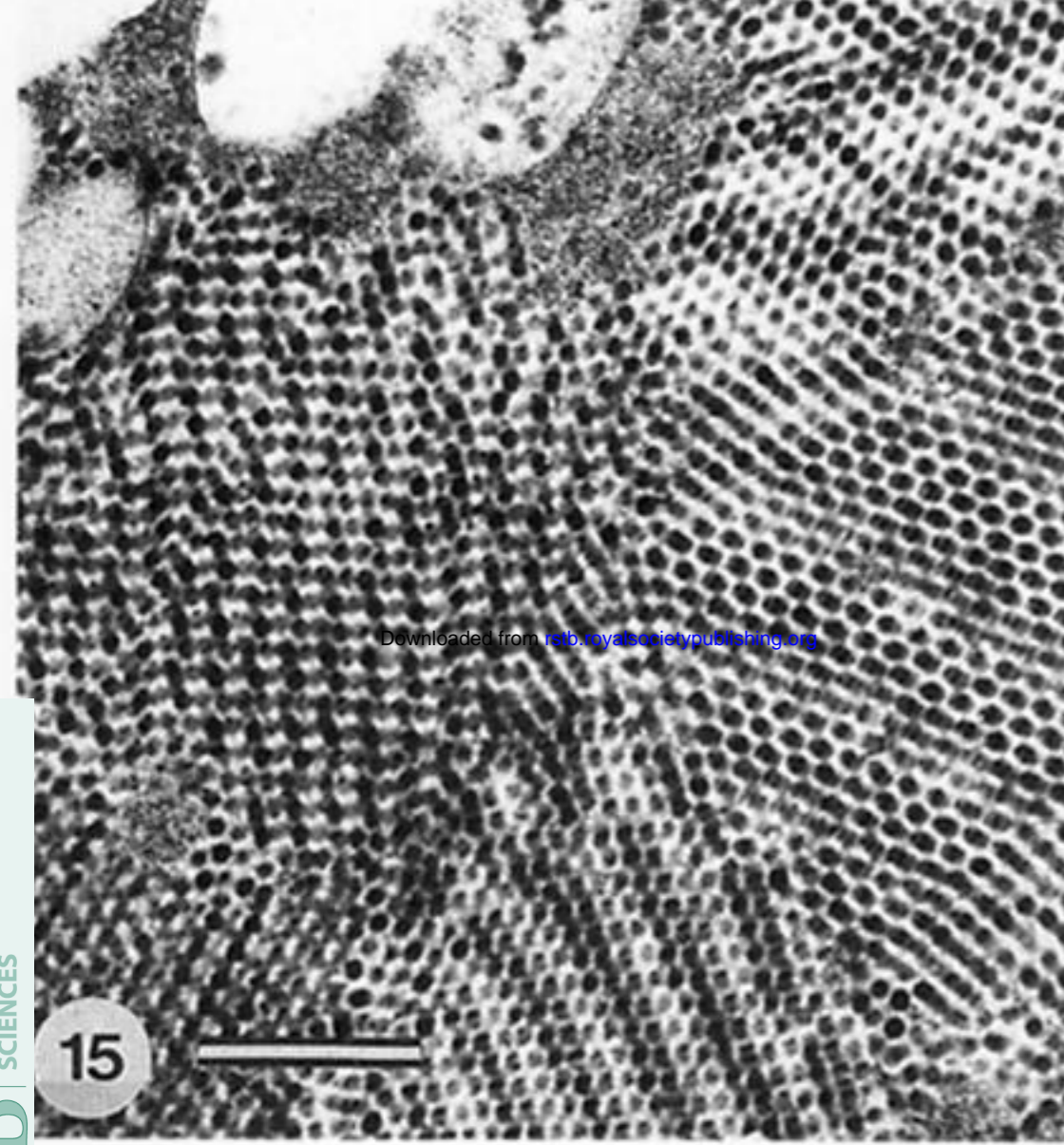
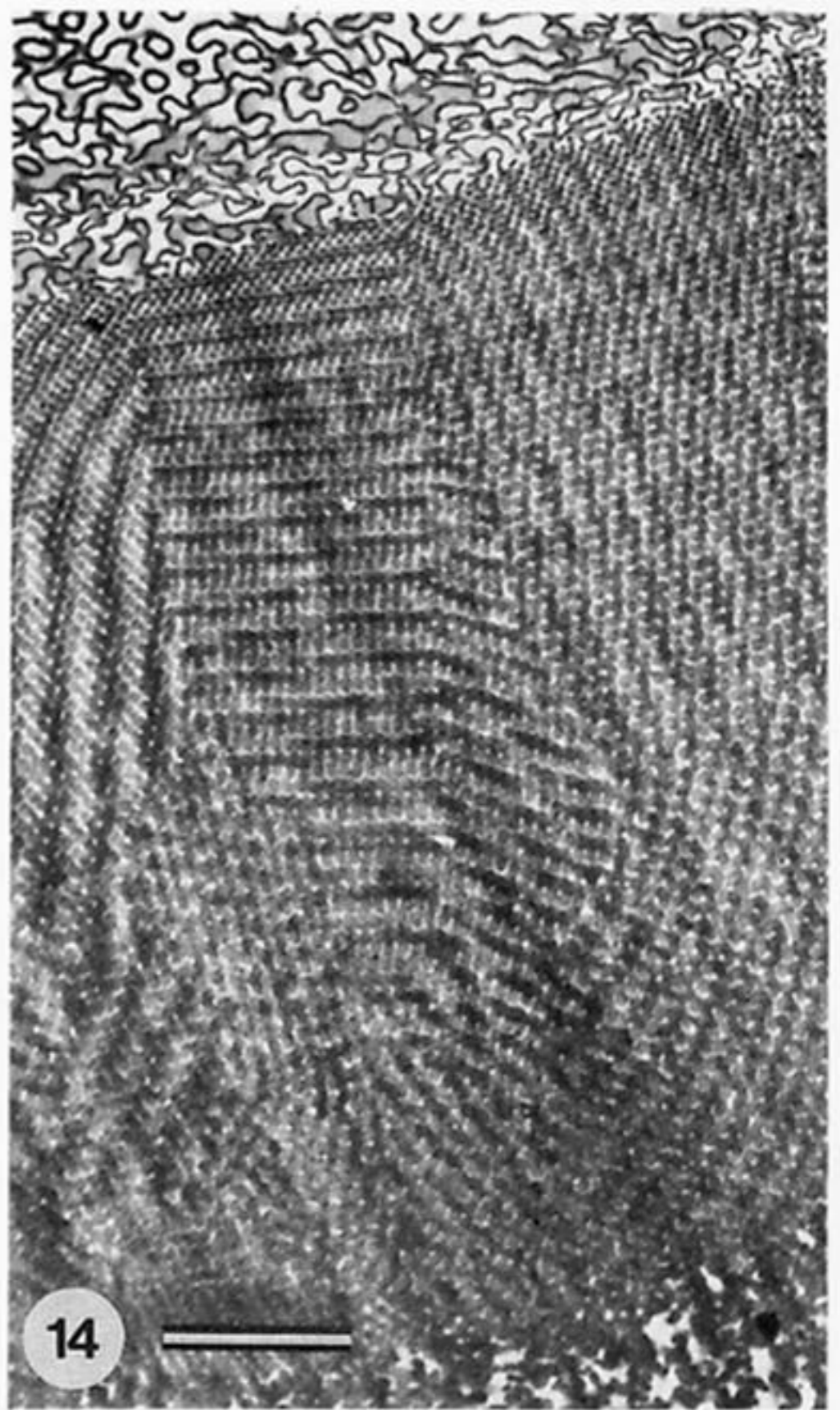
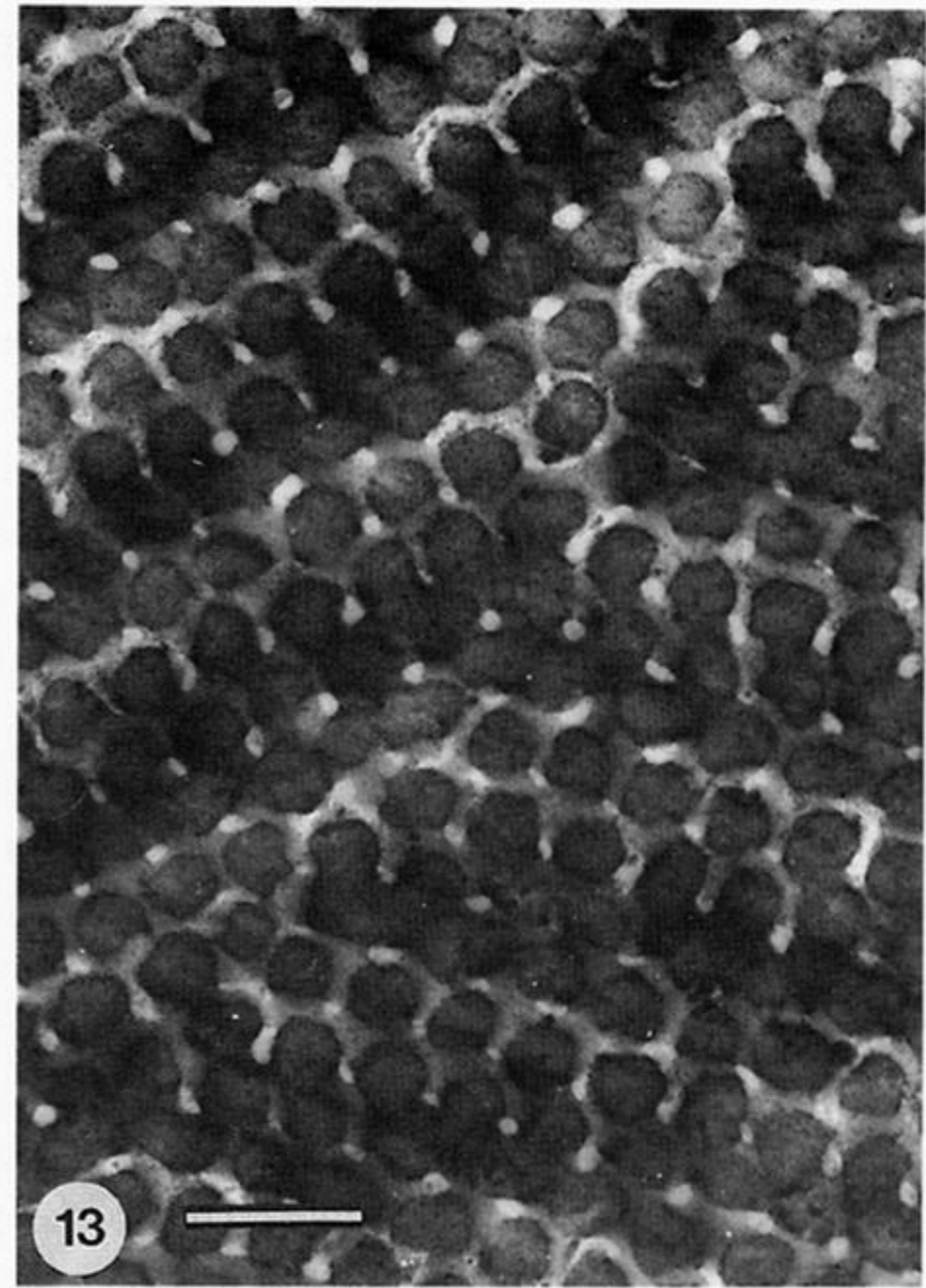
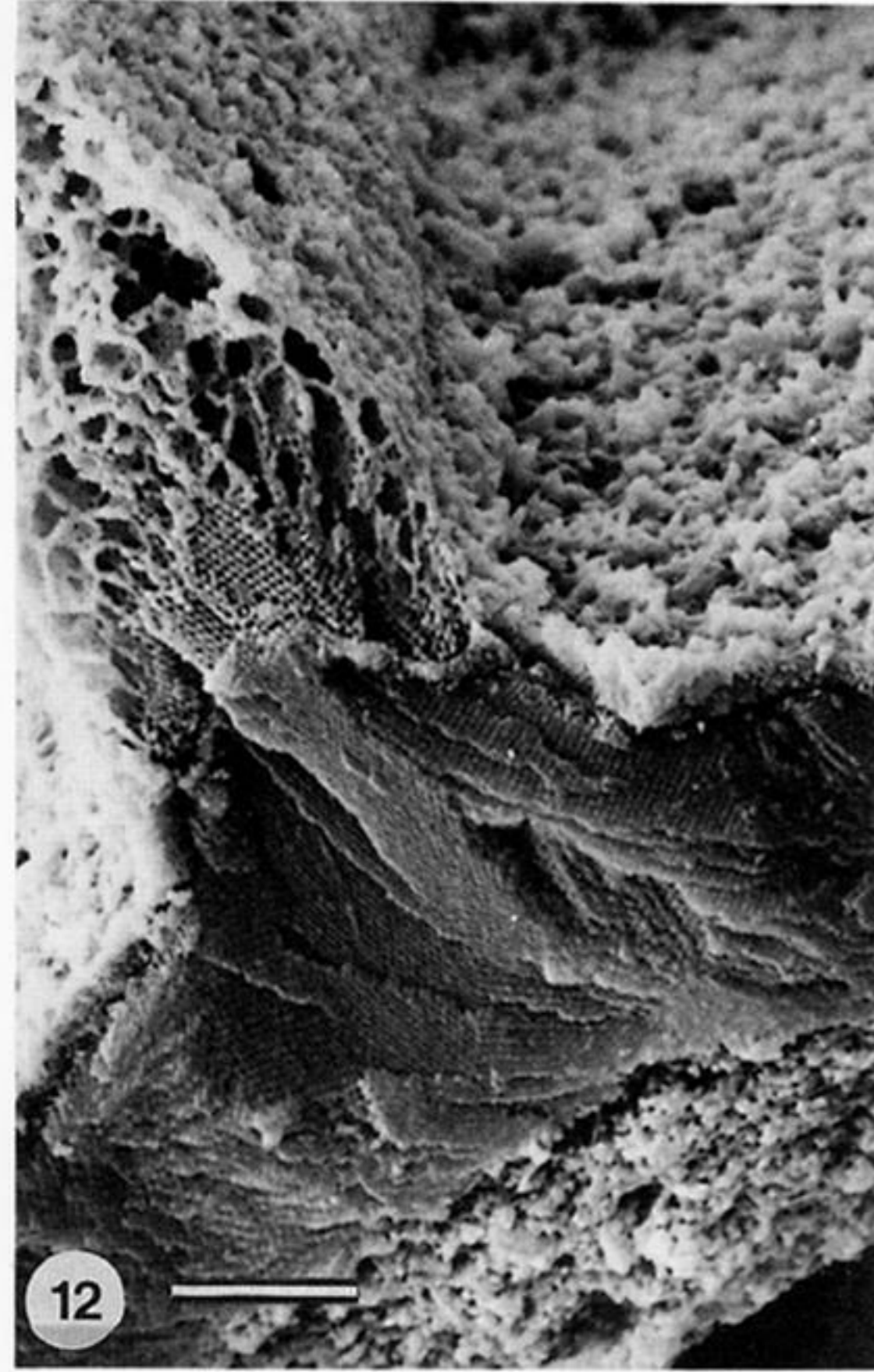
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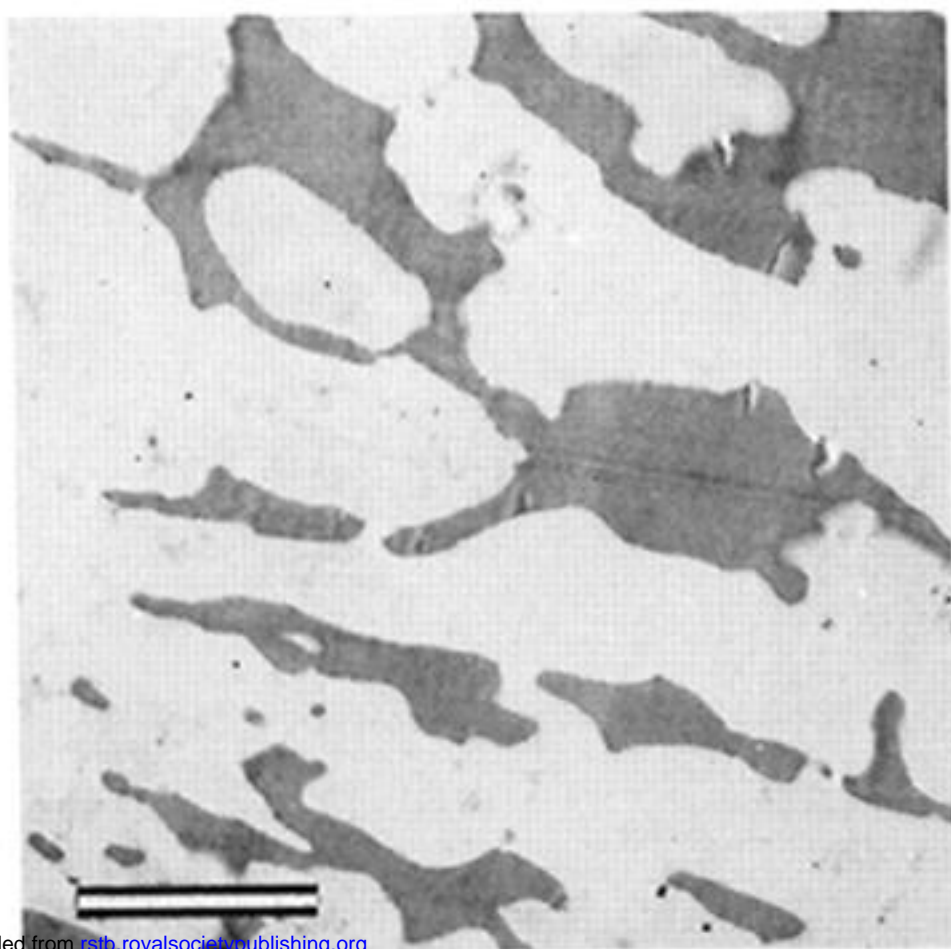
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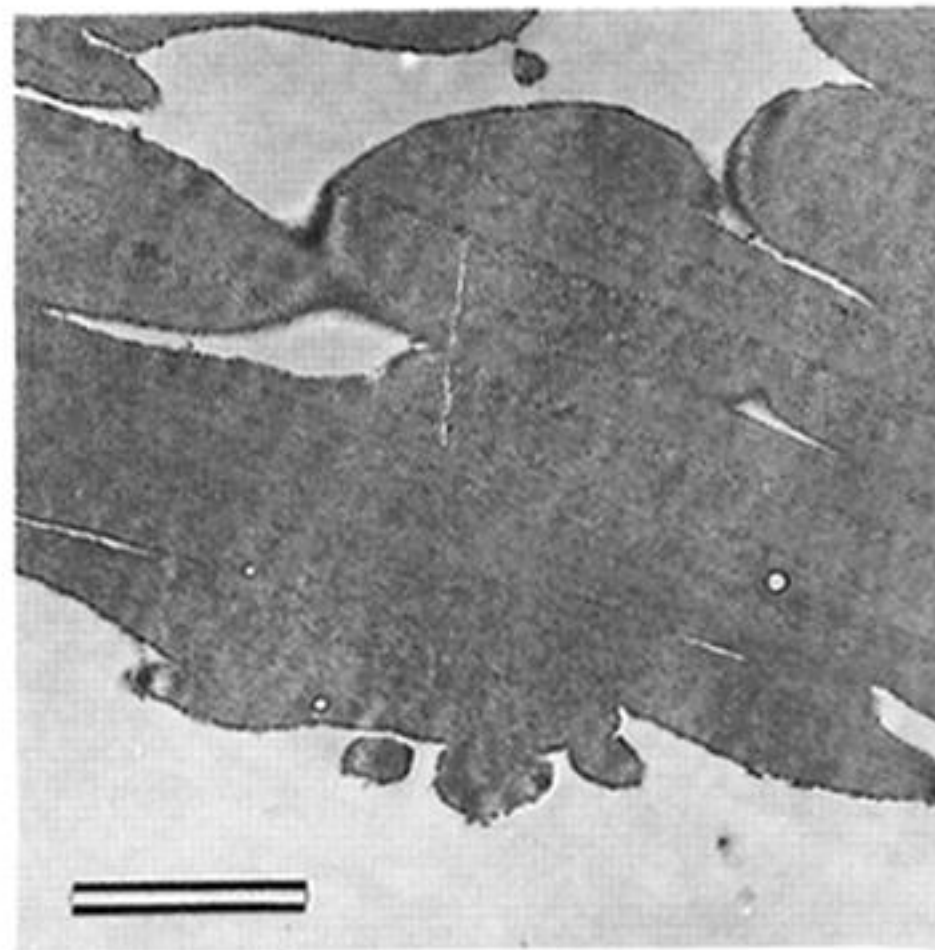
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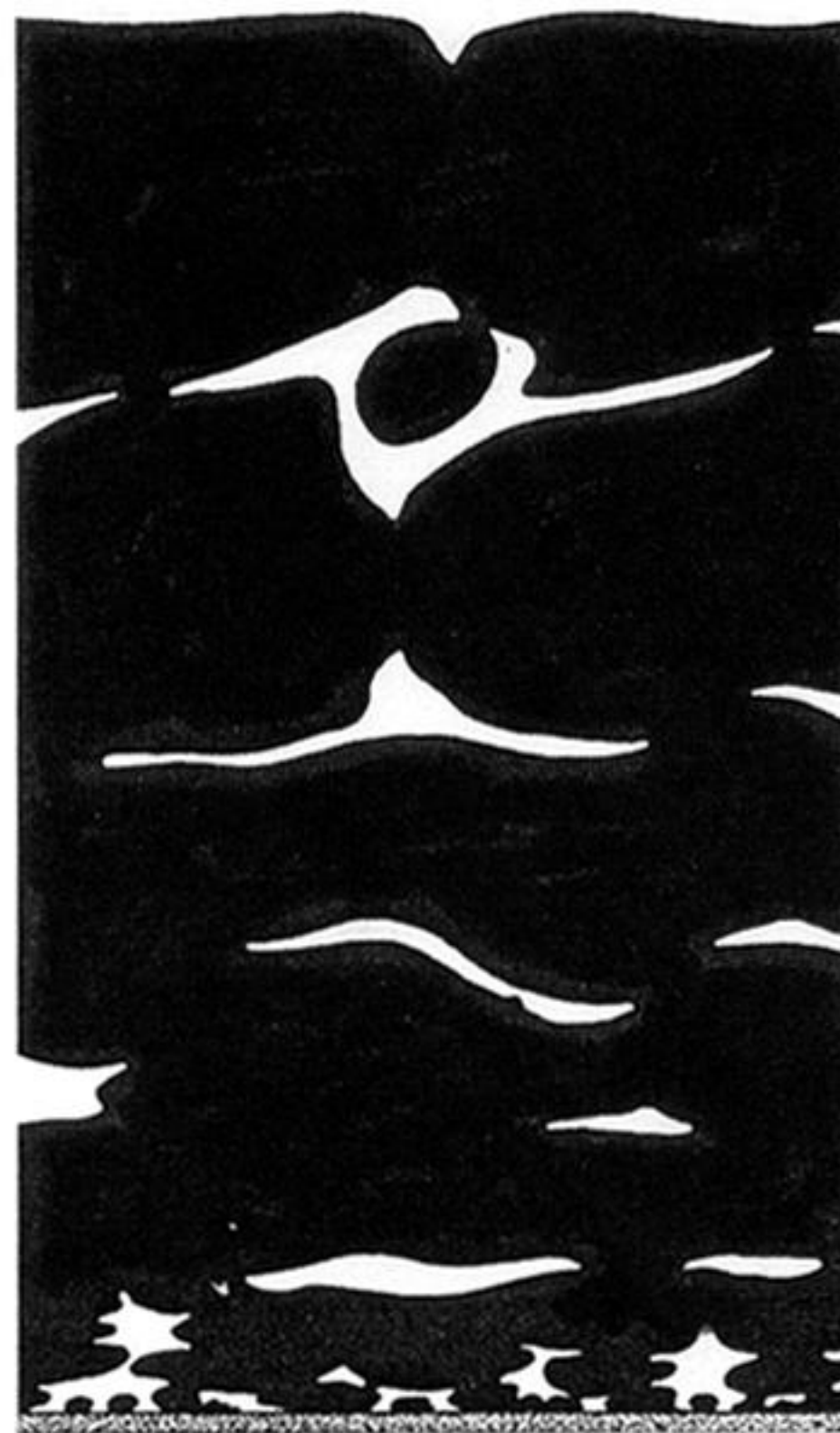
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Lagenicula crassiaculeata



Lagenicula subpilosa f. *major*

Figure 24. Examples of two spore wall types (from related Carboniferous species). On the left, a low density spore wall which is thoroughly opaque due to light scattering at the numerous interfaces between the wall material (black) and the cavities (white). On the right, a wall of similar thickness but in which there are fewer cavities, with the result that the spore appears more translucent. Upper illustrations are TEM sections of the outer portion of the megaspore wall, lower illustrations are diagrammatic illustrations of the entire wall thickness. Scale bar left = 3 μm , right = 1.2 μm .