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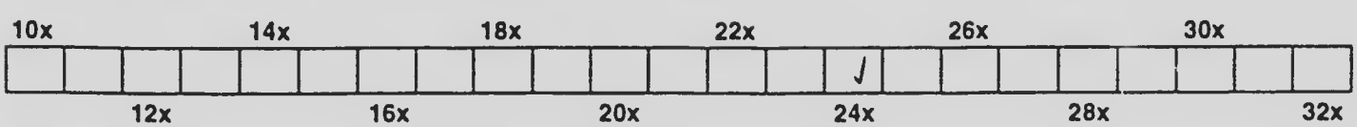

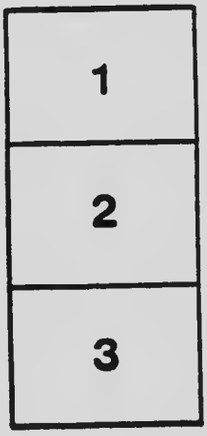

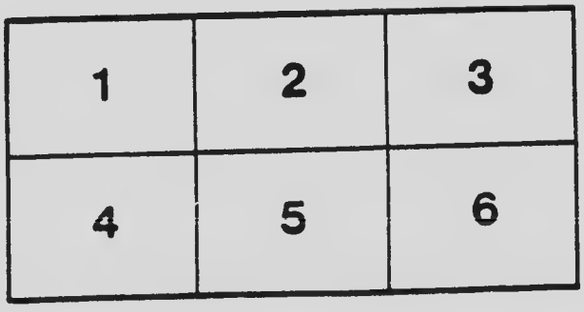

THE MEGASPORE-MEMBRANE OF THE GYMNOSPERMS

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THE MEGASPORE-MEMBRANE* OF THE GYMNOSPERMS

Hofmeister, in his classic comparative researches of 1851, indicated the fundamental connection between the ovule of the gymnosperms and the sporangium of the higher cryptogams. He showed the homology of the embryo-sac of the former with the free spore of the latter in words which deserve prominence in connection with the present work (Hofmeister,[1] p. 141): "Der Embryosaek der Coniferen lässt sich betrachten als eine Spore, welche von ihrem Sporangium umschlossen bleibt." Mettenius,[2] Solms-Laubach,[3] Scott[4] and Worsdell[5] have directed attention to the cryptogamic nature of the foliar and pedunicular bundles of the gymnosperms, thus carrying out in another direction the generalization which Hofmeister reached in the case of the ovule. The occurrence in palaeozoic strata of a group of forms, designated by Petonié[6] the "Cycadofilices," forms which combine pteridophyte and gymnosperm features, and some of which have been recently shown to be seed-bearing (the so-called Pteridospermae[7]), indicates the close connection that existed in past times between these two phyla. The discovery within the last few years of antherozoids in the cycads and *Ginkgo* by Hirasé,[8] Ikeno,[9] Webber,[10] and Lang,[11] affords more evidence of the relationship of the gymnosperms to the vascular cryptogams.

The investigation with which the present account is concerned deals with another of the cryptogamic features of the gymnosperms. A megaspore-membrane has for a long time been somewhat generally recognized as occurring in certain forms of this group of primitive seed-plants. The extent of its occurrence and the character of the coat in the whole group have, however, not as yet received the attention they deserve, in view of the comparison made by Hofmeister in 1851, as indicative of the free-sporing ancestry of the gymnosperms,

* The term megaspore-membrane or megaspore-coat is applied ordinarily only to the coat of the uninucleate spore. It is here used to designate as well the investing coat of the prothallium, undoubtedly the representative of the former which has been delayed in development

and from the phylogenetic importance which attaches to the relative state of development of the membrane in the different groups and subgroups of this division of the spermatophytes. Indeed at the present time, information which will help to indicate the phylogenetic positions, especially of the subgroups of the conifers, is very desirable on account of the balanced state of conflicting testimony from various sources, especially of such as is based on the interpretation of the female "flower," and also because of the lack of historical evidence, a lack which Dr. Scott clearly states in his recent *Fossil Botany* (p. 483): "On the whole, it is impossible, in the present state of knowledge, to say which tribe or family of the Coniferae is the most ancient." It is then chiefly with the object of affording evidence of the relative antiquity of the forms of the Coniferales that this study of the megaspore-membrane is concerned.

In addition to the investigation of the megaspore-membrane, the tapetum has received considerable attention, since a relationship between the two structures was observed during the progress of the work.

Material for the purpose of the investigation was secured during the last two years. Many of the forms required not being native, I could not personally collect material of them and am indebted to the kindness of the following persons for rendering it available : Sir W. T. Thiselton-Dyer, Director of the Royal Gardens, Kew; D. H. Scott, M.A., Ph.D., Honorary Keeper of the Jodrell Laboratory, Royal Gardens, Kew; W. H. Lang, D.Sc., Lecturer in Botany, University of Glasgow; Wm. Trelease, Director of the Missouri Botanical Gardens, St. Louis; J. M. Coulter, Ph.D., Head Professor of Botany, University of Chicago; C. J. Chamberlain, Ph.D., Instructor in Botany, University of Chicago; M. A. Chrysler, Ph.D., Fellow in Botany, University of Chicago; A. A. Lawson, Ph.D., Lecturer in Botany, Stanford University; B. L. Robinson, Ph.D., Asa Gray Professor of Systematic Botany and Curator of the Herbarium, Harvard University; E. W. Oliver, A.M., Instructor in Botany, Harvard University; W. C. Coker,

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In the following account the order in which the forms are taken up is that indicated in Engler and Prantl's "Die natürlichen Pflanzenfamilien."

CYCADALES.

I. *Cycadeae:* Ovules of *Cycas revoluta* at a comparatively early stage of development have a well marked megaspore-membrane. The coat is 4.5 μ thick at the stage when the prothallium consists of a parietal stratum of protoplasm with a single row of numerous nuclei* embedded in it. It consists of two layers (photo. 1, pl. 3), the endosporium† and the exosporium, which are approximately equal in thickness at this stage. The double nature of the coat is, however, usually not apparent in sections from ordinarily prepared paraffin material. The coat withstands the infiltration of paraffin much more than the tissues of the ovule and in consequence is often "dragged" in sectioning. In the "dragged" condition it appears as a single layer with fine granules slightly projecting from its surface. It is variable in thickness but ordinarily about twice as thick as the norm. The true structure of the coat is always apparent in sections from ovules which have

* About 100 nuclei are present in a 5 μ section through the megaspore, and cell formation is beginning in the basal region.

† The endosporium is always towards the left in the figures, next the prothallium.

been subjected to prolonged infiltration of paraffin (m p. 56°C.) and cut 2 to 5 μ thick at a low temperature. In such preparations the endosporium and the exosporium are quite distinct from each other, a clearly defined line of demarcation occurring between them. Along this line in the sections a separation of the layers sometimes occurs for a short distance (middle of photo. 1, pl. 1). Moreover, the two layers of the coat are different from each other in structure, and hence the double nature of the coat is the more apparent (fig. 1, pl. 1). The exosporium is granular towards its somewhat irregular external border and a fine radial striation can be made out in this part, while towards the inside it becomes gradually more homogeneous. The endosporium appears in section to be sub-divided into two longitudinal strata, by a granular area (fig. 1, pl. 1). The outer of these strata is very finely granular and yellow in colour in the unstained condition, as is the exosporium, while the inner is quite homogeneous, translucent and almost colourless. The zone separating the two sublayers is indistinctly defined, the area in question presenting the appearance of being radially and somewhat coarsely granular at its centre, gradually merging into the strata on either side of it. In very rare instances I have observed that, in the sections of the coat, the two strata of the endosporium are torn from one another at places along the granular area. The endosporium besides being quite different from the exosporium in structure, expands much more than the latter in certain fluids. A tension is thus set up between the layers which, in sections, results either in their separation, or in a "coiling" of the whole coat. The former occurs in cases where the sections of the coat remain attached to the slide (middle of photo. 1, pl. 1), the latter where the sections are detached and float freely in the fluid. In addition, it would seem that the endosporium is more impenetrable to paraffin than the exosporium, since in sectioning the former is often "dragged" and its structure obscured, while the latter is clearly cut.

The double nature of the coat and the structural as well as the chemical differences in its layers are clearly brought

out by the action of stains and reagents. A number of stains were experimented with, but the best results were obtained from the use of a very dilute solution of Ehrlich's acid haematoxylin, with a small amount of alum solution added, followed by a dilute aqueous solution of safranin. The exosporium stains cherry red, the endosporium a variety of colours passing into one another gradually. From dark red along the outer border the endosporium becomes reddish violet, then dark violet along the median granular area, and finally dark and then lighter yellow towards the inner side. Sometimes a tint of orange is seen in the yellow area. The action of the stains on the layers of the coat gives an intimation at least of their chemical composition. A more definite idea of this is obtained by the use of certain standard reagents. Most reliance has been placed on the action of chlorzinc iodine. When thin sections of the young membrane (photo. 1, pl. 1) are treated with this fluid the exosporium becomes yellowish-brown with a tint of red in it, while the endosporium exhibits a variety of colours, one shading into another. The outer stratum of this layer is somewhat darker than the exosporium but very similar to it in colour, while the inner is slightly violet becoming less so towards the inner side. It is usually more pink-violet than the walls of the ordinary parenchyma cells of the ovule. Between the two differently coloured strata of the endosporium is a narrow greenish-yellow area which shades gradually into those on either side. The boundary line between the endosporium and the exosporium is dark and distinct after treatment with chlorzinc iodine, and the coat is somewhat swollen by the fluid and appears less granular. After the action of iodine and sulphuric acid upon sections of the membrane, the outer layer becomes dark yellowish-brown, while the inner has a yellowish-brown outer stratum which entad passes into light yellow, greenish-yellow, and finally into blue. Within the blue can often be seen a homogeneous inner greenish-yellow margin into which the blue shades. From the well known action of th stains and reagents used it is evident that we have to do with a coat whose exosporium is suberized but whose

endosporium is of very complex chemical composition. The inner part of this layer contains a substance which seems closely related to pectin.* Towards the median area of the endosporium this substance is largely replaced by cellulose. The cellulose in turn, in the outer stratum of the endosporium, gives place gradually to suberin, the outer border of the layer being constituted chiefly of the latter.

In older ovules of *Cycas revoluta* the megaspore-membrane is still distinctly double but it has increased considerably in thickness. At the stage of prothallial development when cells are forming (two layers thick in this case) the membrane is of quite uniform distribution around the prothallium and slightly more than 5 μ thick. The increase in thickness does not affect the two layers equally but is chiefly confined to the endosporium which at this stage is about one and one-half times as thick as the exosporium. The latter layer appears more distinctly radially striated in cross-section than in the case of the younger membrane. Sections of it cut parallel to the free surface of the coat are very coarsely granular. The granules are dark yellow in colour but differ from one another both in size and in form. They appear to be surrounded by irregular spaces. The exosporium would thus seem to be formed of little columns or fibrillae which are radially arranged and so responsible for the appearance of radial striation in cross-sections of the coat. This interpretation of the structure of the exosporium is verified by the character of the cleavage of the layer. When sections are broken across the cleavage plane is always at right angles to the free surface of the coat and along the line of the striation. Sometimes two breaks in the exosporium occur near one another and a small rectangular mass is torn out of the layer. This cleavage feature of the coat is characteristic of the younger membrane of *Cycas* as well, though the latter is not so frequently found broken. The inner layer of the megaspore-coat, as stated above, is much thicker at this stage than at the previous one. It pre-

* This part, as was stated, does not colour a decidedly orange-yellow in safranin (Strasburger [12], p. 135), but a rather dark yellow. At a later stage, however, to be described next, the orange-yellow is more distinct.

sents clearly the appearance of being subdivided into two strata. These, however, vary considerably in relative thickness at different places in the sections, but the outer portion is always thicker than the inner, sometimes appearing as thick as the exosporium. The boundary between the two strata of the endosporium is, as in the young condition, a somewhat radially granular part. Moreover, a slight difference in the chemical composition of the endosporium is to be noted at this stage. A greater portion of the outer stratum of the layer is completely suberized, while its inner part gives a more decided indication (orange yellow in safranin) of the presence of pectin, the cellulose being more definitely confined to the median part of the layer at this than at the previous stage. The coat "coils" more readily also in the older condition, "coiling" being an especially marked feature at this stage in sections which have been treated with potash solution. The endosporium swells very much in this fluid and the coat may attain a thickness of 8 μ.

In addition, there is present on the inner side of the coat a thin layer which is in very intimate association with it, so intimate in fact that it is readily mistaken for a part of the coat. It is, however, formed of the outer walls of the superficial prothallial cells. This apparent "reinforcement" of the coat may be very considerable in older ovules. Indeed in ripe seeds of many forms a *suberized* band is present over the free surface of the prothallial cells which is as thick or even thicker than the coat itself and is difficult to distinguish from it, since the two structures are alike in chemical composition and often closely in contact with one another.

The chief change in the megaspore-membrane of *Cycas revoluta* at the stage when the archegonia are formed is in the relative thickness of its two layers. The exosporium is fully double as thick as the endosporium, though the thickness of the whole coat is but slightly increased. The exosporium is more plainly radially striated than at previous stages.

I obtained only a single ovule of *Cycas Rumphii* for examination. It was at a stage of development similar to the

earliest examined of *C. revoluta*, i.e., when the prothallium is about to begin cell formation. At this stage the coat of *C. Rumphii* is so similar in structure, in thickness, and in chemical composition to that of *C. revoluta* as to be indistinguishable from it. In fact had not the shape of the ovule been different in the two species I should have been unable to distinguish the sections of them.

In order to determine whether the structure and chemical composition of the coat of the megaspore of *Cycas* has been affected by the retention of the spore in the megasporangium it seemed desirable to study the coat of the microspore for comparison. Material of *Cycas* was not available, but a study of the coat of the mature microspore of *Pinus resinosa* was made instead. The coat in this form is about 5 μ thick in the region above the prothallial cells, its exine and intine being about equally thick and separated by a dark line. The outer layer of the coat is roughly radially striated and the inner homogeneous. When thin sections (3 to 5 μ thick) have been treated with chlorzinc iodine the exosporium is yellowish-brown and the endosporium shades from light violet on the inside to darker violet about the middle, then into greenish-yellow, and finally into light brownish-yellow along the outside. In safranin the intine is somewhat orange yellow on the inside and pink towards the middle and outer parts. The exine is cherry red. In haematoxylin the inner and outer borders of the intine stain less intensely than the median part. It is thus seen that the strata of the intine of the microspore of *Pinus* react similarly with stains and reagents to those of the endosporium of *Cycas*, though they are not so distinct from one another as are those of the latter. The enclosed and the free spore coats are thus in structure and in chemical composition fundamentally alike.

For the purpose of comparison of the process of suberization in spore-coats and in cell-walls an examination was made of the suberized walls of the epidermal cells on the upper surface of the megasporangium of *Zamia integrifolia*, a form whose megaspore-coat will be described later. When sections

are treated with chlorzinc iodine, around the protoplasm of these epidermal cells is a uniformly thick homogeneous layer which is violet in colour. Over the free surface of the cells is a dark brown band from which characteristic wedge-like projections pass inwards between the outer parts of the violet walls of contiguous cells. The dark brown of this band and its projections never comes into intimate contact with the violet of the inner layer but is always separated from it by a greenish-yellow area just as in the inner layer of the spore-coats described above. This colour is no doubt produced from a blending of the colours of the adjoining strata, so that it would appear that we have here to do with an area of composite chemical character, a transitional area between strata of different chemical composition. The suberized walls of epidermal cells are undoubtedly formed from within, and since the endosporium of the coat of *Cycas* consists of similar chemical strata and has a like transitional area between the cellulose and the suberized layers, it seems probable that it, too, is formed from the inside. Confirmation of this view is indicated in the action of chlorzinc iodine on the two suberized parts of the coat. The outer stratum of the endosporium is darker brown in colour than the exosporium and so according to the well established colour reaction of this reagent is of more recent formation.

The present work on the Cycadeae has demonstrated the occurrence of a megaspore-coat in the subgroup and has determined its structure, thickness, and chemical composition. The coat is double, its outer layer suberized and composed of radially arranged fibrillae, while its inner is fairly homogeneous and formed of strata which, though they differ fundamentally in chemical composition, are yet not distinct but pass by gradual change of chemical components insensibly into one another.

The tapetum of *Cycas* at the youngest stage of prothallial development described above consists usually of a single layer of large somewhat cubical cells, on the inside of which there may often be seen here and there some more or less

collapsed ones (fig. 1, pl. 1). The distribution of the tapetum is uniform around the prothallium at this stag just as is the megaspore-coat. The large cells have one to several nuclei in each, two being not uncommon in a section. In the cells are irregular-shaped grains which in stained preparations and in those treated with chlorzinc iodine, etc., appear as vacuoles (fig. 1, pl. 1). These grains in iodine solution* colour at first a brownish-pink or purple, some of them even a red brown. On heating the colour fades, and on cooling and applying fresh fluid the brown colour does not return but is replaced by pink to bluish-purple. These grains are then not glycogenous as at first seemed probable but consist largely of amylodextrin (Strasburger[12], pp. 82 and 83). They are quite different from the starch grains of the nucellar and integumental tissues, the latter being much smaller and giving the characteristic starch reaction. The tapetal cells at the later stage of prothallial development when cell formation has begun are devoid of amylodextrin and their protoplasm appears to be fairly uniformly distributed. The most interesting point, however, with regard to the tapetum in the present connection is apparent when sections of it and of the nucellar tissue are treated with chlorzinc iodine. All the megasporangial cells, even the very thin-walled ones of the more or less collapsed tissue immediately adjoining the tapetum, have violet-coloured walls, while those of the contiguous large tapetal cells are dark yellowish-brown. The walls of the tapetal cells are thus suberized and so afford a striking contrast between this tissue and that of the nucellus. On the other hand the suberization gives indications of an association between the tapetal cells and the megaspore itself, which, to say the least, suggests very forcibly a common origin, and is thus almost in the nature of a demonstration of the sporogenous character of the tapetum.

II (a). *Zamieae-Stangerieae.* In some very young ovules of *Stangeria paradoxa*, the only form included in the Stangerieae

* The solution used was made up as follows: 5 c.g. iodine and 20 c.g. potassium iodide were dissolved in 15 c.c. of water.

the megaspore-nucleus had already divided several times, probably about eight free nuclei being present in the somewhat parietal protoplasm of the young prothallium. Around the protoplasm at this early stage there is present an undifferentiated, glassy-looking thickening which represents the young megaspore-membrane.

At a later stage, when a cellular prothallium has been formed and the ovules are about ready for pollination* the megaspore-coat is a very conspicuous feature. It is about 4.5 μ thick and is very distinctly double (photo. 2, pl. 3), in fact it is very similar to that of *Cycas revoluta* at the stage when the prothallial cells are forming. The exosporium is fairly granular and very deeply and distinctly striated. The endosporium consists of two homogeneous bands, the outer of which is thicker than the inner. These have a narrow granular area between them just as in the case of *Cycas*. The coat, too, is "reinforced" from within by the outer walls of the superficial layer of prothallial cells, so that it appears to consist of four layers, a pair of inner narrow and a pair of outer relatively broad bands. Haematoxylin stains both layers of the endosporium, the outer less than the inner but still to a considerable extent. Safranin stains the outer stratum also and colours as well the exosporium. The outer part of the endosporium is intensely stained as a result of the double staining, and a good contrast is obtained between the layers as is shown in photograph 2, plate 3. The thickness of the inner part of the endosporium appears less in the photograph than its actual thickness, because of the over-staining of the outer part of this layer and its consequent encroachment on the inner. There is also but little indication in the illustration that the inner lighter part is of a composite character, since the "reinforcement" from the cell-walls is very closely associated with the inner part of the membrane. In sections treated with chlorzinc iodine, however, this is evident, since the cell-walls are coloured yellow while part of the endosporium is blue-violet. The transition

* For the material of *Stangeria* at this stage I here express my especial indebtedness to Dr. Lang of Glasgow University, who was kind enough to send me several embedded ovules.

area from the violet to the yellowish-brown (tinted red) of the outer part of the endosporium is greenish-yellow as in *Cycas*. This outer part of the endosporium is homogeneous and coloured similarly to but darker than the finely granular and radially striated exosporium. When sections of the coat are treated with iodine and sulphuric acid the inner part of the endosporium is greenish-yellow to blue in colour and shades as in *Cycas* into the dark yellowish-brown of the outer parts of the coat.

Dr. Lang has represented the coat of *Stangeria*, in his work on the ovule of this form, as a single, thick, somewhat granular band (Lang[11], fig. 16, pl. 17), at a stage of prothallial development similar to that at which I have described it above. I find that the coat of *Stangeria*, like that of *Cycas*, "drags" very frequently in sectioning, and that in this condition it appears as an unstratified, fairly homogeneous layer, much thicker than in normal section, fully as thick as Dr. Lang has represented it. Dr. Lang was primarily interested in other important features and possibly his sections, like many of mine, while showing the structure of the tissues perfectly, gave but a false impression of the coat.

The megaspore-membrane of *Stangeria* thus is similar in every respect to that of *Cycas*, It is double, its endosporium subdivided into two more or less homogeneous strata. The exosporium and the outer stratum of the endosporium are suberized, the latter containing a little cellulose, while the inner part consists largely of this substance with which some pectin is probably associated. Between the two strata of the endosporium is a transitional area, transitional both in structure and in chemical composition, just as in the case of *Cycas*. In thickness, however, the coat of *Stangeria* is somewhat less than that of *Cycas* even when the latter is at a younger stage of prothallial development.

Dr. Lang has described the tapetum of *Stangeria* in several stages of development. His account of it when the prothallium has become cellular is as follows (Lang[11], p. 287) : "Around the megaspore a layer of cells was present which is

clearly to be traced to the sporogenous group. The thick zone of sporogenous tissue present in earlier stages has, however, become reduced to a single layer. . . The cells of this persistent layer (fig. 16) are very large, and stand with the longer axis at right angles to the surface of the megaspore. Sometimes a single nucleus is present; often two are found in each cell." This layer is said ultimately to disappear in the seed. In infertile ovules Dr. Lang has further observed that the tapetum is well developed. My own observations on the tapetum in *Stangeria* agree, so far as they go, with Dr. Lang's description. In the young condition when the nucleus of the megaspore has undergone probably three successive divisions the tapetal tissue is of considerable thickness. The walls of its cells have not, however, as yet differentiated chemically, colouring greenish-yellow in chlorzinc iodine. At the older stage at which the megaspore-membrane has been described the tapetum consists of a single, or at rare intervals of a double, layer of cells. These have thin but yet distinctly suberized walls, just as in the case of *Cycas* at a similar stage of development. In sections of infertile ovules of the same age as the fertile ones described above the tapetum is a very prominent structure, being four to five layers of cells in thickness. Its cells, too, are large and have very abundant protoplasmic contents, while those of the surrounding tissue are in a more or less collapsed condition. Further, the walls of the tapetal cells are thick and suberized while those of the adjoining nucellar tissue are thin and composed of cellulose. The greater development of the suberized tapetum in ovules which develop no prothallium would seem to be in keeping with Dr. Lang's view that the single-layered tapetum, which is characteristic of the older stages of fertile ovules of *Stangeria*, results from the gradual disintegration and absorption by the growing prothallium of the inner cells of the originally many-layered "sporogenous group" surrounding the uninucleate spore. The sharp line of chemical distinction between the walls of the large exterior tapetal cells and those of the adjacent collapsed cells of the nucellar tissue affords evidence of a

similar character, while in addition, by laying emphasis upon the definiteness of the boundary between these tissues, it brings into more intimate association the tapetal cells and the megaspore, affording thus clearer evidence of the sporogenous nature of the tapetum.

II (b). *Zamieae-Euzamieae*. *Dioon imbricata* at a stage of prothallial development previous to the appearance of archegonia has a well developed coat about 3.8 μ thick and very distinctly double. The exosporium is about one and one-half times as thick as the endosporium, and the fibrillae of which it is composed are quite regularly radially arranged and coarse. The endosporium presents the appearance of being subdivided into two strata, the outer thicker than the inner, but both quite homogeneous. The inner stratum of the endosporium stains with haematoxylin, while the outer as well as the exosporium are coloured by safranin. In chlorzinc iodine the exosporium and the outer part of the endosporium are yellowish-brown, the former a little lighter than the latter. The inner part of the endosporium is pinkish-violet and passes by a darker coloured, somewhat granular narrow portion into the yellowish-brown of the outer part of the layer. There does not seem to be any shade of red in the suberized part of the coat when it is treated with chlorzinc iodine, as has been noted in the case of *Cycas* and *Stangeria*, but this may be due to the, unfortunately, poorly preserved material examined.

Warming in 1879[14] gave an illustrated description of the mature coat of the same species of *Dioon* that I examined. He states that the coat is layered like that of *Ceratozamia robusta*, whose megaspore-membrane he described and figured two years previously. There is, however, a great difference in his figures of these forms, two layers more being represented in *Ceratozamia* than in *Dioon*. (Compare Warming,[13] fig. 23, pl. 3, and Warming,[14] fig. 7, pl. 6.) The statements, too, in the layering of the coat in the résumé and in the original (Danish) of Dr. Warming's article do not agree (Warming[13], résumé p. 18, and the Danish p. 100). Yet it seems probable that the coat of both forms is considered double since this is

the statement c. its structure in the Danish and since, also, Warming's statement on the cycads in general (*Handbuch der Botanik*, p. 168) would lead us to so regard it. Further, Dr. Warming states that the outer part of the coat of *Dioon* appears to be made up of a large number of small "prismatic bodies," structures which are apparently considered the homologues of certain "fusiform crystal-like structures," stated to be common in other tissues of *Dioon*. It is in this way that Dr. Warming accounts for the radial striation of the outer portion of the coat. Undoubtedly the "prismatic bodies" correspond to the little columns or fibrillae which I found present in the coat of *Dioon* and other forms. The structures I have observed are, however, too irregular to be termed prismatic though they have something of a hexagonal outline in cross-section, and in this respect correspond somewhat to the representation of them that Dr. Warming has given (Warming,[14] fig. 6, pl. 6). The coats of *Dioon* and *Ceratozamia* are considered as completely suberized, colouring dark yellow in chlorzinc iodine. I find that the inner stratum of the endosporium contains no suberin. In fact in chemical composition the coat of *Dioon* is very similar to that of *Cycas* and *Stangeria*, the inner stratum of the endosporium giving a decided reaction for the presence of cellulose, while its outer stratum and the exosporium are suberized.

Dr. Warming has figured a younger stage of *Dioon* in which the membrane is represented as a single homogeneous layer. I have not examined any material of *Dioon* at a similar stage but it seems probable that the coat does not differ from that of other cycads that I did examine (See *Cycas*, photo. 1, pl. 1, and *Zamia integrifolia*, pp. 17-18).

Several stages in the development of the megaspore-coat of *Zamia integrifolia* were examined. When the prothallium is in a comparatively early stage of free nuclear* division a distinct coat about 3 μ thick is present around the parietally placed cytoplasm. The coat "drags" very readily in section-

* I counted as many as 16 nuclei in one section through the centre of the prothallium. This section was not more than 5 μ thick.

ing but appears distinctly double in well prepared sections. Its inner half in unstained sections is transparent and homogeneous, while the outer is yellow, opaque and somewhat granular especially towards the exterior. Moreover, there is present a very dark and granular line about the middle of the coat. Though the general appearance of layering is evident in sections of the coat yet the boundaries of its strata and substrata are less clearly defined than in the case of any other cycad coat that I have examined. In sections stained in haematoxylin and safranin the inner and somewhat irregular border of the endosporium is light pink in colour and exterior to this is a homogeneous violet band which externally becomes darker, its outer boundary being formed by an intensely dark granular line which separates the coat into two equal parts. Exterior to this central line is a violet-pink band slightly granular in appearance and stained more intensely than any other part of the coat except the line above referred to. Apparently continuous with this deeply stained part is one stained a lighter pink. It is more granular than the latter and indications of a radial arrangement of the granules can be detected. This part probably represents a poorly differentiated exosporium about one-third the thickness of the whole coat. In chlorzinc-iodine the inner margin of the coat is light greenish yellow and after several days' treatment separates from the coat as a thin film. The greenish-yellow is succeeded externally by a yellowish violet which in turn is replaced by dark green when the median granular area is reached. Exterior to the middle line of the coat is a dark yellowish-brown band, neither border of which is clearly defined. It passes on the outside into the lighter yellowish-brown of the exosporium.

At a stage when about two layers of cells are present in the prothallium, the megaspore coat of *Zamia integrifolia* has increased somewhat in thickness and the layers are more differentiated. The exosporium constitutes about one-half of the coat and is more distinctly granular and radially striated than it is in the younger condition. The endosporium is composed of two strata and has an addition to its thickness

from the walls of the outer prothallial cells. The so-called "reinforcement" is similar in colour when the sections of the coat are treated with chlorzinc iodine to the inner light yellow border of the younger coat. Like the latter, too, after prolonged treatment it separates from the coat. The inner stratum of the endosporium is violet in chlorzinc iodine and the outer, which is about double as thick, is brownish yellow, as is the exosporium. The boundary line between the exosporium and the outer part of the endosporium is more clearly marked than in the younger coat.

At a later stage, when cell formation is well advanced in the prothallium the megaspore coat of *Zamia integrifolia* is 3.8 to 4 μ thick. The exosporium at this stage is thicker than the endosporium even with its "reinforcement" which has increased considerably. The outer layer of the coat, too, is more distinctly radially granular.

In mature seeds of *Zamia integrifolia* I found that the coat is quite thick, 4.5 μ at least, without the addition from the cell-walls. The latter has differentiated into an inner "cellulose" and an outer "suberous" layer so that when the coat is in contact with the prothallial cells it is difficult to [illegible] out the inner cellulose layer of the endosporium even when sections are treated with chlorzinc iodine. The whole endosporium at this stage is very much reduced in thickness constituting only between one-fourth and one-sixth of the whole coat. The exosporium is plainly radially striated, the fibrillae of which it is composed being quite fine and somewhat irregularly radially arranged.

The tapetum in the mature seed is represented merely by some suberized débris from collapsed cells. In the younger stages it is very similar to that of *Cycas* and *Stangeria*, being formed of a jacket of cells tolerably uniform in thickness, investing the prothallium. The cells have suberized walls which are more or less spongy-looking, as if they were irregularly radially perforated. Usually they are uninucleate and contain some small amylodextrous grains. The number of layers of cells constituting the tapetum varies at different times.

The material of *Zamia floridana*, at a stage when fertilization is about to take place, showed a megaspore-membrane which in section is very similar to that of the other species and about 4.2 μ thick. The endosporium and the exosporium were not so unequal in thickness as in the case of *Z. integrifolia*.

At a stage when the archegonia have been fully formed, *Ceratozamia longifolia* has a megaspore-membrane which is 4.5 μ thick in the lateral and basal parts of the ovule. In the archegonial region, however, it thins out fully one third. The exosporium is coarsely radially striated and fully three times as thick as the endosporium. It has a very thin homogeneous inner portion and the boundary line between it and the endosporium is distinct.

Warming,[13] as already stated, has described the coat of *Ceratozamia robusta*. His figure indicates that it is in appearance very like that of *C. longifolia*. The coat he describes is mature, however, and is represented as much thicker (by my computation from his figure) than the younger coat of the latter species that I examined. The coat of *C. robusta*, too, is said to be suberized, while I found the one of *C. longifolia* not different in this respect from that of the other cycads.

The megaspore-membrane of the Cycadales in all the forms examined shows the same fundamental characteristics of structure and chemical composition. The coat is double, its outer layer, especially in the later stages of development, being composed of distinct, radially arranged fibrillae. This layer is suberized in all cases. The endosporium presents the appearance in cross-section of being composed of two more or less equal and homogeneous longitudinal strata. These differ in chemical composition, suberin, cellulose, and pectin replacing one another gradually from the outside to the inside of this layer. The character of the succession of the chemical strata of the endosporium and the transitional nature of the areas between them have led to the conclusion that the coat is formed from within, that the endosporium is in fact the formative layer of the coat. This conclusion gains support from the fact that as the exosporium increases in thickness,

the endosporium decreases, the former apparently at the expense of the latter. With regard to the increasing distinctness of the striation of the exosporium which keeps pace with its increase in thickness, it may be possible to consider it as an indication of a progressive degeneration resulting from physiological activity. The development of the striation is at least associated with the growth of the prothallium and the accompanying transfer of food-material from the surrounding cells to it. No matter what view is taken of the mode of formation and differentiation of the coat, its uniformity in structure and in chemical composition in the Cycadales as a group has been demonstrated. Moreover, the coat is thickest and best developed in the Cycadeae, and thinnest in the Euzamieae, while in the Stangerieae it is of an intermediate character.

The tapetum is uniformly well developed in the Cycadales judging from the representatives of its subgroups that I have examined. It is evenly distributed around the prothallium and in certain cases is as much as four to five layers of cells in thickness. In all forms it probably thins out in the later stages of ovular development, being practically eliminated from the mature seed, though even here its pre-existence is indicated by certain suberized remains. In all the forms that I have examined the tapetal cells have suberized walls. The suberization it would seem possible at first sight to regard as a cenogenetic feature developed in relationship to the function of the tapetal cells, whose office it is to supply nutrition to the growing megaspore. The study of various tapetal layers, however, shows that the cells constituting such layers may have cellulose walls (*infra* pp. 25-26). Again if such were the case it might reasonably be expected that the suberization of the tapetal cells would not be so marked in infertile ovules where no megaspore is developed. As has been observed in the case of *Stangeria* suberization is even more marked in such cases. Thus suberization of the walls of tapetal cells would appear to be a palingenetic feature and to afford almost a demonstration of the sporogenous nature of this tissue. The plurinucleate condition of some of the cells

constituting the tapetum in the various forms and the course of development of this tissue as indicated by Dr. Lang's work on *Stangeria* affords further evidence of a similar nature.

GINKGOALES

As early as 1855 there is a record of the occurrence of a megaspore-membrane in *Ginkgo*. Hooker and Binney[15] state that "an extremely delicate membrane (fig. 16) surrounds the albumen of Salisburia." It is represented in their figure by a line. Williamson,[16] in 1876, referred to the coat in the same connection but with no more detail of its structure. Recent investigators seem to have overlooked it entirely. In fact in Seward and Gowan's[17] résumé of the knowledge of *Ginkgo* (up to 1900) there is an indirect negation of its presence. They say: "The upper part of the endosperm is covered by a thin papery membrane which represents the crushed remains of the nucellus."

On embedding and sectioning some mature seeds I found that this "thin papery membrane" is of a composite character and represents not only the "crushed remains of the nucellus" but tapetal débris and the megaspore-membrane as well. The megaspore-coat in fact is in a fair state of preservation in the mature seed (fig. 2,* pl. 1). It is very distinctly double and 4.5 to 5 μ thick, thinning out somewhat in the archegonial region. The exosporium is from four to six times as thick as the endosporium and composed of quite loose fibrillae which are very irregularly radially arranged, so much so that in sections the outer layer often presents the appearance of a net-work, though the reticulation is more apparent in oblique sections than in accurately transverse ones. Staining differentiates the inner part of the endosporium from the other parts of the coat. The former takes the haematoxylin stain while the rest of the coat stains in safranin. Under the action of chlorzinc iodine this inner part becomes violet and the outer part of the endosporium yellowish brown. There is present in *Ginkgo* as in the cycads a greenish yellow but very narrow

* Because of technical difficulties I have not represented the stratification of the endosporium in *Ginkgo* and many other forms.

transitional area between the strata of the endosporium. The exosporium is yellowish brown, the reticulation being very distinct in this fluid. The outer walls of the superficial prothallial cells are very thick and consist of a suberized outer layer and a cellulose inner one, the former about one-half as thick as the latter. Where the coat abuts directly on the suberized part of the cell wall it is difficult to distinguish between the "reinforcement" and the coat itself.

When the megaspore of *Ginkgo* is in the parietal nucleated condition, at about the same stage* as that of the younger ovule of *Cycas* (photo. 1, pl. 1) described before, its coat is quite thin, about 3 μ thick. It is double, as in the mature seed, but the exosporium is not more than one and one-half times as thick as the endosporium. The former is plainly striated at its outer border but becomes less and less so towards the endosporium, and finally blends almost imperceptibly with the outer stratum of the latter. The endosporium is formed of two chemically different strata, as the staining and treatment with chlorzinc iodine indicates. Haematoxylin lays hold of the immature coat more strongly than of the mature one and more readily obscures the layering of the coat, especially of the endosporium. The action of chlorzinc iodine on the membrane at this stage is similar also to that on the mature coat. A thin film, of the same colour as the protoplasm in chlorzinc iodine, extends along the inner side of the coat sometimes in contact with it but usually separated from it in the sections. It may be a part of the megaspore-coat or the beginning of the outer walls of the first row of prothallial cells which will shortly be formed. It is, however, characteristically present, and adds about .3 μ to .4 μ to the thickness of the coat.

At a very early stage of nuclear division in the megaspore of *Ginkgo*, when possibly sixteen free nuclei have been formed, there is a slight thickening around the protoplasm in which

* In a section about 5 μ thick through the centre of the megaspore I counted over 80 nuclei, while not more than 100 are present in *Cycas* at the stage indicated in photo. 1, pl. 1.

the nuclei are embedded. In chlorzinc iodine this young membrane is coloured light yellow, possibly with a shade more of green in it than the protoplasm to which it adheres.

The megaspore-coat of *Gingko* is thus very similar to that of the Cycadales. It is quite uniformly distributed around the prothallium in both groups, and in structure and chemical composition presents essentially the same features, though the endosporium of *Ginkgo* at the stages I examined does not seem to be so thick as that of the cycads.

The tapetum in *Ginkgo* at the youngest stage described above is four to five layers of cells in thickness. The inner cells are square to oblong in longitudinal sections of the ovule and have less dense contents than the outer ones, which are somewhat elongated tangentially. When the megaspore has many nuclei in a parietal stratum of protoplasm (at the intermediate stage described above) the tapetum consists of a jacket one to several cells in thickness. The cells have fairly dense contents and the walls though not well developed are suberized. Some of the cells, too, are plurinucleate. When the seed is matured the tapetum is represented merely by some suberized débris around the prothallium (fig. 2, pl. 1). The tapetum of *Ginkgo* like its megaspore-membrane is not so well developed as it is in the case of the cycads but undoubtedly it is an homologous structure, originating from the sporogenous tissue.

CONIFERALES.

I (a). *Pinoideae-Abietineae-Araucariinae* : In the posthumous account of *Gnetum* by William Griffith,[18] which appeared in 1859, there is a reference to the megaspore-membrane of *Agathis*, one of the two genera constituting the Araucariinae. We are told simply that this structure, which he termed the "amnios" and of which he probably did not recognize the homology, forms a very distinct lining to the cavity in the "nucleus" (nucellus) of *Agathis*. This is at a comparatively early stage of prothallial development.

In the youngest stage of *Agathis Australis** that I have examined, the megaspore has about 16 nuclei in a 10 μ longi-

* Material of this form has become available recently through the kindness of Professor Treub and Dr Valeton

tudinal axial section. At this stage the megaspore-coat is very variable in thickness, averaging possibly 4.5 μ. It has a somewhat granular inner stratum and an outer one about equally thick but hyaline in appearance. There is no sharp line of distinction between the strata of the coat though the inner stains in haematoxylin and the outer in safranin. In chlorzinc iodine the inner stratum is violet, light yellowish towards the inside and darker towards the outside. The latter portion passes by a greenish-yellow area into the slightly brownish-yellow outer part of the coat. Around the coat there are the remains of a suberized tissue, in intimate contact with it. Possibly its presence may indicate that a suberized tapetum was present at an earlier stage. Numerous pollen-tubes (6–10), entering the apical region of the nucellus, converge irregularly around the megaspore, some passing even deeper than the latter which occupies the middle half of the longitudinal axis of the sporangium. The pollen-tubes and the megaspore resemble one another very closely at this stage, so closely that in cross-sections it is difficult to distinguish them unless a number of consecutive sections are examined. They are alike in size, similar in contour and irregularly arranged in the axial portion of the sporangium. Their walls, too, are closely associated with the surrounding tissue and in structure and in chemical composition are very similar. The similarity amounts almost to an identity in certain of the cross-sections, since numerous nuclei and much protoplasm are present in the pollen-tubes at this stage.*

Around the megaspore at the young stage described above and filling up more or less the areas between the pollen-tubes are groups of cells very full of starch grains and protoplasmic contents. This tissue undoubtedly supplies nourishment to

* Six or seven nuclei are not uncommon in a tube at this stage, while in one I counted as many as thirteen. This recalls the interesting condition recently described by Juel[19] in *Cupressus Goveniana*, where, however, not a nuclear but a cell-complex is present in the pollen-tube, derived in this case from the body cell. A detailed account of the pollen-tube in *Agathis*, and of certain other peculiar features to which my attention was directed in the course of the present work, will appear in a subsequent paper.

the prothallium and must be considered as a tapetum. It differs very markedly, however, from the tapetum in the cycads and *Ginkgo*. Its cells are uninucleate and their walls composed of cellulose while the tissue itself has no sharp external boundary but passes by easy transition stages into the ordinary nucellar tissue, being merely a differentiated inner part of the latter.

At a later stage just after fertilization has been effected, the coat does not present even so definite structural features as it does in the younger condition. It is much thinner and colours more yellow in chlorzinc iodine. The prothallium is relatively very large at this stage, much of the surrounding nucellar tissues having been absorbed. A few of the inner layers of the latter are starch-bearing but more collapsed than in the younger condition. Remains of pollen-tubes are apparent especially in the apical region of the prothallium. In the ripe seed of *Agathis* the coat is still present. It is fairly thick in some places but usually thin and much disorganized. The protiallial cells of the mature seed have very thick cellulose walls and over the superficial ones a suberized band extends, which is, however, clearly distinct from the coat. In infertile ovules collected at the same time as the ripe and fertile ones, the prothallium has collapsed, though the integument and nucellus are nearly as fully developed as in the fertile ones. The shrunken prothallium is surrounded by the megaspore-coat which closely invests it. This structure is undifferentiated so far as layering is concerned, but fairly uniform in character. In chlorzinc iodine the bulk of the coat is yellow to brownish-yellow in colour, the inner border only being violet.

The coat of the megaspore of *Agathis* is thus poorly differentiated structurally at all stages. In the young and better developed condition it resembles very closely the wall of the pollen-tube, though it is slightly thicker than the latter. Indeed so very close is the resemblance that the numerous pollen-tubes surrounding it are readily mistaken for infertile megaspores. The resemblance of the megaspore-coat of *Agathis* to the intine of the microspore of *Pinus*, both in struc-

ture and in chemical composition, is very intimate. This becomes even greater when the microspore has shed its exine and grown down into the tissues of the nucellus, for the wall of the pollen-tube (intine) of *Pinus* is then of increased but more variable thickness. The megaspore-coat of *Agathis* never differentiates any farther, but in the later stages becomes even less definite in structure and finally is much disorganized in the mature seed. As it becomes older the composition changes gradually, the cellulose being almost completely replaced in the later stages by a substance resembling suberin. The latter gives, however, not so dark a brownish-yellow in chlorzinc iodine as ordinary suberized layers.

The functioning tapetum of *Agathis* differs very decidedly, as has been noted, from that of the cycads and *Ginkgo*. It is clearly of nucellar origin and in this respect may be distinguished as a secondary tapetum, that of the cycads and *Ginkgo*, which is regarded as sporogenous, being considered as of a primary nature. The latter is represented in *Agathis* only by suberized remains.

The coat of *Araucaria imbricata* is very variable in thickness at the stage indicated in photograph 3, plate 3, and is closely applied to the surrounding tissue. In this young condition it is in appearance very like that of *Agathis* at about the same or possibly a little later stage of development. It is much thinner, however, and in composition has a larger proportion of cellulose in it. A careful examination of mature seeds of *Araucaria Brazilicnsis* and of *A. imbricata*, as well as of infertile ovules of the former, failed to reveal the presence of even a trace of the coat. The coat is then much less developed than that of *Agathis* though undoubtedly similar in character to it. In both the characteristic outer suberized layer which is found in the cycads and *Ginkgo* is absent and the coat resembles the wall of the pollen-tube both in structure and in chemical composition. It is suggestive, in this connection, that the wall of the megaspore of the Araucariinae grows in intimate association with the surrounding tissues, just as that of the pollen tube does.

Around the megaspore of *Araucaria* in the younger stages (see photo. 3, pl. 3) there is a mass of tissue of elliptical outline which represents the tapetum. Its cells contain many starch (amylodextrous) grains and their walls are suberized. The tissue is chiefly aggregated around the base of the megaspore, being very thin along the sides and fairly so at the apex. At places its suberized cells shade gradually into the surrounding cellulose-walled cells of the nucellus, while at other parts they are distinctly marked out from them. The tapetum of *Araucaria* is very different from that of *Agathis*. In the latter it is of fairly uniform distribution while in the former it is massed in the basal region. In *Agathis*, too, the only suberized part consists of collapsed cells within the functional tapetum, while nearly all the tapetum of *Araucaria* has suberized cell walls. The tapetum, however, in each form looks as if it were an integral part of the nucellus, merely a differentiated inner portion of its tissue.

I (b). *Pinoideae-Abietineae-Abietinae* : An idea of the distribution and prominence of the megaspore-membrane in this subgroup may be gained by a glance at photographs 4 to 10 (pls. 3 and 4). The coat is thick in the chalazal region and thins out gradually towards the micropylar portion of the prothallium, being not more than one-third as thick at the apex as at the base of the megaspore.

The coat of *Pinus resinosa* (fig. 3, pl. 1) at the stage indicated in photograph 4, plate 3, just prior to fertilization, is about 4.2 μ thick at the lateral basal region of the prothallium from which part the drawings have usually been made. It is distinctly double, the endosporium being about one-third as thick as the exosporium (fig. 3, pl. 1). The former is homogeneous and appears as in the cycads to be subdivided into two longitudinal strata, the inner, in the case of *Pinus*, only about one-half as thick as the outer. The former stains in haemotoxylin while the rest of the coat takes the safranin stain. In chlorzinc iodine the inner stratum of the endosporium is violet while the outer is yellowish-brown, considerably darker than the exosporium. The fibrillae of the latter are more or less

collapsed and irregularly radially arranged, as indicated in the figure. The tapetum of *P. resinosa* at this stage is almost confined to the basal part of the ovule and consists of uni or pluri nucleate cells which are more or less broken down. Their protoplasmic contents are sparse and their suberized walls irregular and somewhat porous, like those of the cycads but yet much thinner. It is rather a striking feature with regard to this primary tapetum that it thins out with the megaspore membrane, thickest at the base and almost disappearing around the apical region of the prothallium. *P. strobus* differs from *P. resinosa* in having a more delicate megaspore-coat and one which is more evenly distributed around the prothallium. The coat at the stage just subsequent to fertilization is not more than 3.8 μ thick. Its endosporium is nearly as thick as its exosporium. The latter is so irregular and "ragged" along its outer border as to give one the impression that the decrease in thickness may be due to the destruction of the outer part of this layer. The tapetum like the megaspore-coat is fairly evenly distributed around the prothallium, disappearing only above the archegonia. It consists of a jacket of partially collapsed cells with suberized walls which lie away from the megaspore-coat at a considerable distance. In the intervening space some granular material is found. Outside the tapetum is a layer of very loose open cells which are not compressed as in most other cases. *Pinus sylvestris* and *P. Austriaca* have coats and tapeta which at the stage when the archegonia have been fully developed are similar to those of *P. resinosa*.

The coat of the larches differs from that of the pines in its distribution. In both *Larix Europaea* (photo. 5, pl. 3) and *L. Americana* there is scarcely a trace of the megaspore-coat in the archegonial region. In the former the thinning out process is somewhat abrupt while in *L. Americana* which has a longer prothallium it is more gradual, the latter occupying an intermediate position in this respect between the European species and the pines. The coat of these two forms is somewhat thicker and coarser than that of *Pinus*. Its endosporium

does not constitute more than one-sixth to one fifth of the whole coat (figs. 4 and 5, pl. 1) and consists of two substrata which are more nearly equal in thickness than in *Pinus*. The exosporium is very regularly formed and the fibrillae composing it are coarse, especially in the case of *L. Americana* (fig. 5, pl. 1). The distribution of the tapetal cells agrees with that of the megaspore-coat. In the chalazal region of the prothallium there are a few cells with suberized walls while around the rest of the gametophytic tissue there are only scattered traces of the tapetum.

The megaspore-membrane of the spruces examined is quite similar to that of the larches at the same stage of development. It is slightly thicker, however, and is not so attenuate in the micropylar region. Photographs 6 and 7, plate 3, are of *Picea nigra* and *Picea excelsa* respectively and indicate the distribution of the coat around the prothallium. Figures 6 and 7, plate 1, illustrate the structure of the membrane in the lateral region of *P. excelsa* and in the basal region of *P. nigra*. The thickness of the membrane in our black spruce is not quite so great as that of the European species. Both are quite thick, however, though the endosporium is extremely thin in each. *Picea alba* has a coat which is possibly a little thinner than that of *P. nigra* but otherwise like those of the other species.

The megaspore-membrane of *Tsuga Canadensis* looks very thick, as photograph 8, plate 4, shows. It is, however, not so thick as it appears. Some granular material is massed against it and makes its outer layer appear thicker at a low magnification than it really is. In figure 8, plate 1, this material is represented as slightly removed from the coat, which in thickness and general character resembles that of *Pinus resinosa* very much (cf. figs. 3 and 8, pl. 1). The hemlock, however, has a thinner endosporium than *P. resinosa* and its exosporium is somewhat more regular.

Abies balsamea photo. 9, pl. 4 has a well formed megaspore-membrane which is of about the same thickness as that of *Picea nigra*. Its endosporium is very thin and its exosporium thick and coarse (fig. 9, pl. 2). All the membranes of the Abietinae so

far described have been from ovules at approximately the same stage of development, about the time of fertilization. They are all quite similar to one another as is indicated in figures 3 to 9 (pls. 1 and 2). The membranes are "reinforced" on the side next the prothallium by the adjacent cellulose walls of its superficial cells. Two areas are recognized in the endosporium of all forms, an inner one which stains with haematoxylin and becomes violet in chlorzinc iodine, and an outer one which is stained by the safranin and colours dark brownish-yellow in chlorzinc iodine. The exosporium is finely or coarsely fibrillar in all. It stains in the safranin and is coloured a lighter yellowish brown in chlorzinc iodine than the outer part of the endosporium. The tapetum at this stage is distributed much as is the megaspore coat. It consists of at least a single layer of loose cells at the base of the prothallium and thins out towards the apical region. The cells have suberized walls and some of them at the base are plurinucleate as well. In these respects they resemble the tapetal cells of the cycads and *Ginkgo*.

In the mature seed of *Pinus ponderosa*, which is very large, the megaspore membrane is 4.5 to 5 μ thick. The exosporium is five to six times as thick as the endosporium and consists of fibrillae which are fine but which look at places as if they were more or less disorganized. They are always much pressed together and seem almost to have lost the radial arrangement which characterized them at an earlier stage. In chlorzinc iodine there is a violet inner border to the coat but the body of it is yellowish brown. The mature coat of *Pinus Banksiana*, whose seed is small, is quite thick, 4.2 μ. Its endosporium is thin and homogeneous and the exosporium is coarsely and irregularly granular. In chlorzinc iodine the coat is yellowish-brown with a violet inner border. *Pinus insignis* is very similar to the former in the size of the seed and in the character of the megaspore-coat. The exosporium is, however, somewhat finer and retains more of the radial arrangement of its fibrillae. Ripe seeds of *Cedrus Atlantica* have a coat which in structure, thickness, and in chemical composition is almost

identical with that of *Pinus ponderosa*. The size of its seed is, however, much less. The megaspore coat in the ripe seed of the European larch is slightly thinner than that of *Cedrus*, but otherwise similar to it. From the comparative uniformity of the thickness of the megaspore-membrane of the above mentioned forms it would seem that there is no direct relationship between the size of the ovule and the thickness of the coat, though possibly the larger seed has slightly the thicker coat. The tapetum in the mature seeds of all the forms examined is entirely disorganized.

At a young stage in *Pinus resinosa* when the prothallial cells are forming (three cells in depth) the megaspore coat is about 3 μ thick and appears quite similar to those of *P. pumilio* and *P. sylvestris* as Mlle Sokolowa[20] has represented them, incidentally, in her work on endosperm formation, as a means of indicating the orientation of the cells. I find that this is true also of *P. sylvestris* at a slightly younger stage (fig. 10, pl. 2). The endosporium is homogeneous and composed of two substrata as in the more mature condition described before. The exosporium is finely and indistinctly radially granular, but only about one and one half times as thick as the endosporium. The exosporium and the outer layer of the endosporium are suberized. The tapetum is better formed and more evenly distributed at this than at the later stage. Its cells are irregularly shaped and one to several nucleated. Their walls are not differentiated but the walls of the cells immediately adjoining them (to the right in fig. 10, pl. 2) are of cellulose. The coat, too, is of uniform distribution around the prothallium at this stage (photo. 10, pl. 4). In very young ovules of *Pinus resinosa*, when about six nuclei are present, in the parietal protoplasm of a section 4 to 5 μ thick through the young prothallium, the only trace of a megaspore-coat is a more homogeneous outer border around the protoplasm. The tapetum is thick but its cells have not acquired differentiated walls. The outer ones are elongated tangentially and quite narrow radially while the inner ones are nearly equiaxial. These cells have a large

[111]

granular nucleus. The whole layer is four to five cells in thickness and the one form of cell gives place gradually to the other. At a later stage when about twelve nuclei are present in a section of the prothallium the tapetum is differentiated into two layers of oblong cells with large open nuclei. The long axes of these cells are directed radially. Similar stages in the development of the megaspore-coat and of the tapetum have been observed in *Pinus sylvestris* and in *P. strobus* and *Larix Europaea*.

The Abietinae have a megaspore-membrane and a tapetum which are very uniform in structure and in the character of their distribution. With respect to distribution both the tapetum and the megaspore membrane are peculiar and strikingly different from the cycads. The reduction of both in the Abietinae towards the micropylar region about the time of fertilization suggests that in these forms this part is the seat of the activities which are adverse to the retention of these structures. Probably the reproductive processes are concerned in this matter since the coat and the tapetum develop quite uniformly around the prothallium until shortly before the time when reproductive activity is at its maximum. The coat, too, in the Abietinae is not so thick even in the basal region as it is in the cycads but in structure and in chemical composition the membranes in the two groups are similar. The tapetum, though very much less developed and more quickly disorganized than in the cycads, is of the same nature, and in the course of its development passes through similar stages to that of *Stangeria* (*supra*, pp. 14-15). The cells, too, composing it are sometimes several nucleate and their walls are suberized. The tapetum of the Abietinae is thus a primary one, derived as in the cycads and *Ginkgo* from the sporogenous tissue.

I (c). *Pinoideae-Abietineae-Taxodineae*: Photograph 4, plate 4, is of *Sciadopitys verticillata*. The gametophytic tissues have shrunk considerably and are widely separated from the tapetum. The latter consists of from four to five layers of more or less collapsed cells which have as yet thin walls and are very full of granular material. The

tapetum in turn has separated from the nucellar tissue. The megaspore membrane adheres to the prothallium and in sections is a very prominent structure. It is double and 4.2 μ to 4.5 μ in thickness, the endosporium being from one half to one-third as thick as the exosporium (fig. 11, pl. 2). At places in the sections these two layers are torn from one another. The endosporium consists of two homogeneous bands with a narrow dark and granular looking area between them, the inner hyaline and the outer light yellow and less homogeneous. The exosporium is light yellow also. It is, however, indistinctly radially striated and finely granular. When breaks occur in the sections of this layer they are always transverse just as in the case of *Cycas*. In fact the coat is very similar to that of *Cycas revoluta* at nearly the same stage of prothallial development. The whole coat is somewhat thinner, however, while its exosporium is if anything thicker. In staining the coat shows a tendency to take up haematoxylin readily and the structure of the endosporium especially is quickly obscured. In chlorzinc iodine the layering comes out very distinctly. The inner part of the endosporium is bluish-violet, lighter towards the interior and dark along the narrow granular area between the strata. The outer stratum is brownish-yellow as is the granular exosporium. When "dragged" the coat in chlorzinc iodine appears as an irregular thick single layer consisting of a mass of dark brown granules in a homogeneous yellow matrix. I have not observed any "reinforcement"* of the coat in *Sciadopitys* though this may be so closely associated with the coat as to escape observation. In distribution the coat is uniform around the prothallium, just as it is in the Abietineae at about the same stage of development (cf. photos. 10 and 11, pl. 4). But it is thicker than the latter at this stage by fully one-half (cf. figs. 10 and 11, pl. 2), and in this respect, and in distribution as well, approaches *Cycas*.

The tapetum in *Sciadopitys*, though thick all around the prothallium, is especially thick in the basal region (photo. 11,

* The inner border of the endosporium is certainly yellowish, but it was not observed to separate from the coat as is the case in *Cycas*.

pl. 4) and thus is suggestive of the much reduced tapetum of the Abietinae at about the time of fertilization when the tapetum in the latter is practically non existent except in the basal region of the prothallium. (Compare photo. 4, pl. 3, and photo. 11, pl. 4). The walls of the tapetal cells turn yellow in chlorzine iodine.

A figure of a young ovule of *Cunninghamia* appears in Dr. Arnoldi's paper on the Sequoiaceae (Arnoldi,[21] fig. 2, pl. 7). The prothallium is represented as in the parietal multinucleate condition and the tapetal tissue ("archesporial tissue" of Arnoldi) is four to five cells in thickness in the basal region, almost as thick, but more disorganized than I found it in the case of *Sciadopitys* even at a later stage. The megaspore-coat is not represented in this nor in any of Arnoldi's figures, nor referred to even in such forms as *Sciadopitys* where I have found that it is very thick. It seems probable then that, since the tapetum, which is evidently of a primary nature, is so well developed in *Cunninghamia*, a fairly thick coat is present, since I have found that there is a correspondence in the state of development of these two structures. Material of *Cunninghamia*, however, was not available for examination.

Considerable attention has been recently devoted to the study of the Sequoias. In young ovules of *Sequoia sempervirens* numerous megaspores, ten to twelve according to Lawson,[22] are found. These acquire thick walls and begin germination. Only two or three develop very far (beyond the first division), and but one of these, growing at the expense of the others, develops a cellular prothallium and later bears the archegonia. This one, previous to the formation of cells, is shown in photograph 12, plate 4, with some abortive megaspores around it. The walls of the megaspores are quite prominent in sections and their close association with the tissues of the nucellus apparent. The large megaspore encroaches irregularly on the nucellar tissue, there being little or no suberized remains present, to indicate the presence of a primary tapetum. Shaw[23] states, however, that a well developed tapetum is present at earlier stages but if such is the case it must be disorganized quickly.

The coat at the stage indicated in the photograph "drags" very readily in sectioning and its thickness and structure could not be as definitely determined as is desirable. It is, however, about 2.5 μ thick and consists of two layers (fig. 12, pl. 2). In chlorzinc iodine the inner border of the coat is violet and exterior to this is a yellowish-brown homogeneous stratum, the outer part of the endosporium. The exosporium is yellowish-brown also but finely and indistinctly radially granular. It is about one and one-half times as thick as the endosporium. The coats of the infertile megaspores seem to consist almost completely of cellulose though they are apparently quite thick.

In *Sequoia gigantea* there is but a single megaspore present. This has no coat in the material I examined, when the prothallium is in an advanced stage of free nuclear division, probably about 128 nuclei being present in the peripheral protoplasmic layer. The outer border of this layer is more homogeneous than the rest of it, and this is the only indication of the presence of a megaspore-coat. In the mature seed of *S. gigantea* the megaspore-coat is thin, about 1.5 to 2 μ in thickness. It consists of a homogeneous inner layer and a coarsely granular outer one. The coat comes into intimate contact with the suberized layer of the superficial prothallial cells and is about equal to it in thickness. Within the latter there is a slightly thicker cellulose layer, the "reinforcement" of the coat being thus much thicker than the coat itself at this stage. When "dragged" the coat appears very thick. It consists of a uniform ground-substance which stains considerably like the cellulose walls of the endosperm cells and has large granules more or less rectangular in outline embedded in it or partly projecting from it. These granules stain like the suberized layer of the cell walls but appear when the high power of the microscope is focussed on the matrix as dark, somewhat rectangular areas in the latter. The tapetum in the younger stage consists of a more or less broken row of cells scattered along the inner border of the nucellar tissue. The walls of these cells are not differentiated chemically, but the tissue probably represents a poorly developed primary tapetum.

With regard then to the difference between the Sequoias which has been shown to exist, at least in the *time* of development of the megaspore coat, it may be said that a certain amount of variability is to be expected in vestigial structures. The difference in the present instance is, however, associated with others, which seemed of enough importance to Arnoldi[23] to warrant a separation of the two species by the revival of an old genus, *Wellingtonia*, for the reception of one of them, though Kraus,[24] who has reviewed Arnoldi's work, thinks that there is not justification for the separation of the two species. I have merely referred to the matter here because of the additional point of diversity afforded by the study of the megaspore-membrane.

Cryptomeria Japonica at a younger stage (photo. 13, pl. 4) than that of the young ovules of *Sequoia gigantea* examined has no trace of a megaspore-coat. A poorly developed tapetum is present, however, but the walls of its cells are not differentiated at this stage. In the mature seed of *Cryptomeria* the megaspore-coat is not so thick as it is in *S. gigantea*, but otherwise is very similar to that of the latter.

In *Taxodium distichum* at a comparatively early stage of free nuclear division a megaspore coat is present, as the figures in Coker's recent work on this form indicates (Coker,[25] figs. 50, 51, and 54, pl. 4). Dr. Coker has also stated that at a later stage, about the time of cell-formation in the prothallium, "the wall of the spore" is "furnished with pits" (Coker,[25] p. 20, and figs. 97, 98, and 99, pl. 7). From his figure of them in surface view they are small perforations of the wall of rectangular to roundish outline. The only material of *Taxodium* that I examined was of mature seeds. The megaspore-coat in these, though much collapsed, is about 2.5 μ thick. It consists of a homogeneous inner layer and an outer coarsely and irregularly radially granular one, about double as thick as the inner. The whole coat, except its inner border which is slightly violet in chlorzinc iodine is suberized. When "dragged" the coat appears as a somewhat thick band, very much like that of *Sequoia gigantea*. With regard to Dr. Coker's statement

(Coker,[24] p. 20) that the coat is pitted, I find that this is very true of the exosporium of the ripe seed, but not of the endosporium. The pits are really not pits but irregular spaces around the fibrillae of this layer, as is the case in all the forms that I have examined. The "dragged" coat does, however, look very much as if it were pitted when examined at a low focus.

Dr. Coker has very fully described the tapetum in *Taxodium* (Coker,[24] pp. 17-20). It consists of large starch-bearing cells in the young condition (megaspore mother-cell stage) which not until later become differentiated from the surrounding cells. When the megaspore is in an early stage of free nuclear division the tapetum is two to three cells thick in the apical and lateral regions, and four to five in the basal part (Coker,[25] fig. 47, pl. 4). At a later stage, but before the formation of cells in the prothallium, the tapetum consists of a single definite layer of well formed cells (Coker,[25] p. 18, fig. 51). This layer consists of collapsed cells when the prothallium has become cellular (fig. 53) and is said to become ultimately disorganized, when the prothallium is mature.

Dr. Arnoldi has described in *Sequoia gigantea*, *Cryptomeria Japonica* and in *Taxodium distichum* tapeta which are similar to the singly layered tapetum that Dr. Coker has figured for *Taxodium*. My own observations verify the presence of such a tapetum in the first two species. I also found some suberized material between the gametophytic and nucellar tissues in the mature seed of *Taxodium*. It thus seems probable that a poorly differentiated *primary* tapetum is characteristic of them all. This is in keeping with the relatively poor state of development of the megaspore-coat in these forms. Reference has already been made to Arnoldi's description of the tapetum in *Cunninghamia*. In *Sciadopitys* both tapetum and megaspore-membrane are thick and very different from those of other members of this subgroup which have been examined. Reference has already been made to Arnoldi's proposed separation of the two species of *Sequoia*, and to the added feature of difference between them which the study of the megaspore-membrane has

brought out. Dr. Arnoldi has also found that *Sciadopitys* is so different from the other Taxodinae as in his opinion to be better removed from them. The great difference in development of the megaspore-membrane and the tapetum in *Sciadopitys* from that which was found to be characteristic of the other Taxodinae examined lends support to Arnoldi's view.

II (2). *Pinoideae-Cupressineae*: In *Biota (Thuja) orientalis*, at a stage (photo. 14, pl. 5) when the young embryos are forming, the megaspore-coat appears quite thick. It is not so thick as it appears, however, since the outer walls of the superficial endosperm cells add much (about one-half) to its apparent thickness (fig. 13, pl. 2). At thickest it is not more than 2 μ at this stage. It consists of two layers, the inner homogeneous and the outer granular (fig. 13, pl. 2), the latter perhaps slightly thicker than the former. In haematoxylin and safranin the bulk of the endosporium stains a very intense pink, its inner border violet. The exosporium is coloured light pink. In chlorzinc iodine the two layers of the coat are yellowish-brown, the inner darker and with a slightly violet inner border. Scarcely a trace of the tapetum is present. The coat is of quite uniform distribution around the prothallium at this stage as the photograph shows. *Thuja occidentalis* (photo. 15, pl. 5 thins out normally,* perhaps a little more than *Biota* in the archegonial region. The coat, too, is not so uniform in thickness at other parts as is the case in the former, nor is its thickness ever so great (usually about 1.5 μ). Even the "reinforcement" of the coat from the prothallial cells is less than in *Biota*. Very little trace of a tapetum is found at this stage, and the megaspore-coat comes directly into contact with the cellulose-walled cells of the nucellus.

In the mature seed of *Biota* the coat is about 2 μ thick, not so thick as is the suberized layer of the superficial endosperm cells. The cellulose part of the latter is thicker again than the suberized zone, so that the "reinforcement" in the mature seed is more than double the thickness of the coat. In *Thuja* the

* In one case the coat appeared thicker in the archegonial region than at any other part. Fertilization had not been effected in this case.

coat is very thin, almost disorganized, when the seeds have matured.

Two species of *Cupressus*, *C. sempervirens* and *C. th[illegible]ides*, have a coat which in ripe seeds is very similar to that of *Biota orientalis* but intermediate in thickness between this form and *Thuja occidentalis*.

Some young material of *Chamaecyparis* sp. (?), when about 16 nuclei are present in a 10 μ axial section of the megaspore, showed only a trace of a coat, very much like that of *Sequoia gigantea* at the young stage described before. The tapetum, too, is but poorly differentiated.

The prothallium of *Juniperus sabina*, about the time of fertilization, has a moderately thick (3 μ +) megaspore-coat (photo. 16, pl. 5). It is fairly uniform in distribution up to the archegonial region where it thins out about one-third. The coat "drags" very readily in sectioning and in the photograph appears much thicker than it really is. When cleanly cut it is seen to consist of two nearly equal layers, the outer granular and the inner homogeneous (fig. 14, pl. 2). The exosporium stains light pink in the safranin and the outer part of the endosporium dark pink, while the inner border of the latter is blue in haematoxylin. In chlorzinc iodine the coat appears dark yellowish-brown, the inner part darker than the outer and with a distinct violet inner border. At a younger stage, when the endosperm cells are forming, the coat of *Juniperus* is somewhat thinner (2.5-3 μ) but uniformly distributed around the prothallium, and otherwise much as it is in the older stage described above. The tapetum at this stage consists of a somewhat loose layer of cells. These are more or less equiaxial but somewhat irregular in outline. They contain from one to several nuclei. In chlorzinc iodine their walls, which are thin, are yellow, while the walls of the cells immediately adjoining them externally are violet.

In the mature seed of *Juniperus Virginiana* the coat is not more than 2 μ thick. As in the younger stages it has a homogeneous inner and a granular outer layer. It is intimately associated with the thick suberized layer of the prothallial

cells. This together with the cellulose layer of their walls is fully two and a half times as thick as the megaspore-coat. When "dragged" the coat appears as a fairly thick band light in colour and with yellow granules partly embedded in it, very similar both structurally and chemically to that of *Sequoia gigantea*, described above.

The megaspore-coat of the Cupressineae in contrast to that of the Abietinae does not thin out gradually towards the micropylar region of the prothallium, but is of more or less uniform thickness up to the immediate neighbourhood of the archegonia. The difference in distribution in the two groups is associated with a difference in the arrangement of the archegonia and possibly to be accounted for on this basis, since, as was referred to in the discussion of the distribution of the coat in the *Abietinae*, the reproductive processes which centre around the archegonia may have to do with the partial destruction of this portion of the coat. The tapetum (in *Juniperus* at least) approximates in distribution to the megaspore-coat. Both structures are less fully developed than in the Abietinae. The coat in the young stages as well as in the mature seeds is much thinner and not so well differentiated and the tapetum disappears earlier in the Cupressineae. The tapetal cells also, so far as I have observed, do not acquire such thick nor such clearly suberized walls as they do in the Abietinae.

II (3). *Taxoideae - Podocarpeae*: In *Podocarpus coriacea** at a stage when the suspensorial cells have elongated and forced the embryo-cells somewhat into the prothallial tissues I could find no trace of a megaspore-membrane, though the material was in a good state of preservation. In chlorzinc iodine the thick outer wall of the superficial prothallial cell is deep blue. There is only a trace if any of a light yellow border to the walls, nor is there present between the gametophyte and the nucellus more than a vestige of suberized substance and no indication whatever of a megaspore-coat. The ovules

* The species is the one from Darlington, S.C., whose gametophytes and embryo Dr Coker[26] investigated, and with material of which he was kind enough to supply me.

of the other species of *Podocarpus* examined, *P. Makoyi*, were in the mature condition, some having viviparous embryos (Lloyd[27]) projecting from the seeds. In this species as in the last I could find no trace of a megaspore-membrane. It is of course possible that a megaspore-coat is present in the younger stages. Since, however, no reference is made to such a structure in the literature on this genus and since I have found, with but few exceptions, some trace of the coat in the later stages of development and even in the ripe seed, where it occurs in the developing ovule, I consider that the coat must be either absent in *Podocarpus* or at least very poorly developed. Again, Dr. Coker refers to the absence in *Podocarpus coriacea* of the tapetum or "spongy tissue" which, he states, is characteristic of so many conifers and which I have found to be correlated in its state of development with the megaspore-membrane. This form then must be considered to have either none or a very poorly developed megaspore-coat and tapetum.

In *Dacrydium laxifolium*, on the other hand, the megaspore-membrane is well developed at the stage indicated in photograph 17, plate 5, a similar stage to that of *Podocarpus coriacea* at which I found no megaspore-membrane present. The coat is 4.2 μ thick and very distinctly double, the endosporium being about one-third as thick as the exosporium. The former is homogeneous while the latter is very irregularly and coarsely striated (fig. 15, pl. 2). When stained there is a dark blue inner border to the endosporium while the rest of the coat is pink. Under the action of chlorzinc iodine this inner border of the endosporium is violet to bluish while the rest of the coat is yellowish-brown, the inner part darker than the outer. The exosporium appears at places to consist of little globules of suberin and to be disorganizing. Some of the appearance is no doubt due to the fact that the material was in the herbarium for four years before it was "revived" and fixed. The "reviving" process, however, seemed to be very successful in this case.

I do not pretend to be able to explain fully the difference between *Podocarpus* and *Dacrydium* with respect to the

megaspore-coat, but wish to point out in the former certain correlated specialized features and in the latter corresponding primitive ones. In *Podocarpus* no "spongy" tissue is present around the prothallium, while in *Dacrydium* the remains of such a tissue are evident in sections of prothallia with the megasporangium intact (fig. 15, pl. 2). That this is a mere coincidence cannot be granted, since in the forms which are known to be primitive, e.g. the Cycadales, and Ginkgoales, there is an association in the state of development of the tapetum or "spongy" tissue and the megaspore-membrane. In addition, in other forms which have no megaspore-membrane the primary tapetum has also been found to be absent or poorly developed, as will be seen in the case of forms to be described next. In certain morphological features of the female "flower" these genera are very different from one another. In *Podocarpus* the ovule is anatropous and has two well differentiated and fused integuments, an inner woody and an outer fleshy one. The fertile scales, also, are united in some species to form a "receptaculum" which becomes berry-like at maturity. In *Dacrydium* on the contrary the fertile scales are not fused and differ but little from the ordinary vegetative leaves even when the fruits are mature. The ovules are never anatropous (orthotropous in *D. laxifolium*) and the outer integument is represented by an "arillus"-like structure which may only partly enclose the inner one but is never united to it, except at the base. The absence of the megaspore-membrane and of the tapetum in *Podocarpus*, the fusion of the parts of the "flower" and the resultant complexity of its structure contrast very strikingly with the more primitive state of affairs in *Dacrydium* where the megaspore-membrane and tapetum are present and the "flower" maintains the distinct individuality of its parts.

II (4). *Taxoideae-Taxeae*: In *Cephalotaxus Mannii* at nearly the same stage as that of the *Taxus* indicated in photograph 18, plate 5, there does not seem to be even a vestige of a megaspore-membrane. The outer prothallial cells in this case have a superficial layer which is yellow in chlorzinc

iodine. This is the only indication of suberized material between the latter and the cellulose-walled cells of the nucellus. Another species of *Cephalotaxus* (an undetermined one) presents features similar to those of the one described above. Mlle. Sokolowa has observed that the megaspore-membrane of *Cephalotaxus* is single and thinner than in the case of any other gymnosperm except *Ephedra*, with which she associates it. Her figure of the megaspore coat of *Cephalotaxus Fortunei* represents it as a single finely granular layer about 1 μ thick, but with indistinct borders. This is at a stage when cells are forming in the prothallium, as we learn from her figures (Sokolowa,[20] figs. 14 and 15, pl. 11).

The material of *Torreya nucifera** that I examined is at an early stage of embryo development. In sections it appears on first sight that a well developed megaspore-membrane is present. Marking out the tissues of the gametophyte from those of the nucellus is a rather uniform band which on treatment with chlorzinc iodine is seen to be suberized. In thin sections which were made to determine the structure of this supposed megaspore-coat, it is evident that the layer consists of an aggregation of fibres. The prothallium encroaches irregularly on the surrounding tissues, and no doubt the band referred to is formed of the walls of cells pressed closely together by the growing prothallium which has absorbed their contents. The cells of the inner layer surrounding the gametophyte are here and there apparently empty though most of them are very densely packed with granular substance and have large nuclei. Two or three layers of cells similar to the latter form with the inner layer a quite distinct but irregular jacket around the prothallium. The outer of these cells contain usually a considerable number of starch grains. This tissue undoubtedly supplies nourishment to the prothallium and is of a tapetal nature. Its cells are, however, uninucleate and their walls are composed of cellulose. They are not fundamentally different from the ordinary cells of the nucellus, though they have more

* Dr Coker, who kindly supplied me with the material, says that it was obtained in the Botanic Gardens of Pisa, Italy, and that the species seems to be *T. nucifera*.

dense contents than the latter, into which at places they pass over very gradually. The tapetum here is thus of secondary or nucellar origin as was found to be the case with the functional tapetum of *Agathis*.

In *Taxus Canadensis* at the stage indicated in photograph 18, plate 5, it is possible that there is a trace of a megaspore-membrane in the basal region of the prothallium. If so, the structure is exceedingly thin. The superficial prothallial cells have their free walls covered by a partly suberized layer which is about .75 μ thick. Beneath it and of about equal thickness is the cellulose part of the wall. The pollen-tube, which is much expanded when it has reached the prothallium, contrasts (see photograph) very strikingly with the megaspore in the development of its wall. The tube wall varies in thickness at different parts from 3.3 to 6 μ. In one quite young ovule of *Taxus* examined two embryo-sacs were present, one of these comparatively large with archegonia developed on it, and the other small with its cytoplasm in a parietal layer and having several nuclei (16 probably). Around the smaller one which would probably remain infertile there is a slight but distinct thickening, while around the other no membrane could be distinguished. This is in keeping with Dr. Scott's recent observation of the difference in thickness between the walls of the fertile and infertile megaspores of *Lepidocarpon* (Scott, [28] p. 299). The later stages of *Taxus* up to maturity of the seed gave no indication of the presence of a megaspore-coat, nor was any trace of a tapetum observed at any stage.

The [illegible] genera of the Taxeae thus present a striking uniformity in the absence or poorly developed state of the megaspore-coat and of the primary tapetum. In this respect they contrast very strikingly with the Podocarpeae, in which group there is much difference in the state of development of these structures. The Taxeae from the present standpoint are to be regarded as a specialized subgroup, while the Podocarpeae contain some specialized and some primitive forms.

GNETALES.

In ovules of *Ephedra vulgaris* Mlle. Sokolowa represents a megaspore-membrane, at a stage when the endosperm-

cells are beginning to form, as a single and finely granular layer about 1 μ in thickness (Sokolowa,[20] fig. 23, pl. 12). In older ovules I found the membrane still present (photo 20, pl. 5). It is difficult to determine its structure, however, since the coat is very closely associated with the suberized walls of the surrounding collapsed and compressed cells of the nucellus. Still in very thin sections it can be made out as an attenuate double layer around the base of the prothallium, while around the upper part it appears more as if it were single, the exosporium not being distinct. The outer part of the coat is suberized and its inner border contains some cellulose, the whole coat in the apical region being largely composed of this substance. No tapetum is present at the stage I examined, but there is a band of the compressed inner cells of the nucellus which is thick in the basal region and thins out gradually towards the apex of the nucellar cavity, being in distribution similar to the tapetum of *Araucaria*, and like the latter also having suberized walls.

Hooker[20] in his paper on *Welwitschia* (1863) states that in this form "the embryo-sac is a delicate membrane" which, when the nucellus has elongated, "is found to have disappeared over the summit of the endosperm." Sir Joseph Hooker compares *Welwitschia* and *Gnetum* with regard to this characteristic of the distribution of the coat. This investigator also states that "the membranous remains of the embryo-sac may often be found on the surface of the nearly mature endosperm." In photograph 19, plate 5, the character of the distribution of the coat is indicated, at a stage soon after fertilization has taken place. The coat is very thin, about 1.3 μ, at the stage examined, but still distinctly double, the outer part granular and the inner homogeneous (fig. 16, pl. 2). No trace of a tapetum is present. The nucellar tissue in the basal region is differentiated, however, into an inner more or less collapsed part (see photo. 19, pl. 5), which if compressed would appear thinner but probably very much like the band that has been described in *Ephedra*.

In Griffith's[18] paper on *Gnetum* (1859) there is a statement that a megaspore-coat is present at a young stage of ovular development but that it disappears in the later stages. Hooker's[29] (1863) statement that the coat of *Gnetum* is similar in distribution to that of *Welwitschia* gives us further information with regard to it. Reference is made in Karsten's[24] paper (1892) which deals with this genus to the presence of a megaspore-membrane around the young spore. Lotzy[30] (1899) has referred to the coats of the megaspores of *Gnetum Gnemon*, "which make them appear much more cryptogamous than the embryo-sacs of most higher plants." The megaspore-membranes are represented as single and finely granular layers. Those of the prothallia which will become fertile are, I have estimated from his figures, about 1.2 μ thick when free-nuclear division is well advanced (Lotzy,[34] fig. 27, pl. 4). Those of the infertile prothallia are thicker, some of them at certain places being represented as fully 2.3 μ thick.

The coat in the three genera of the Gnetales is thus thin and the tapetum poorly developed. In distribution the coat is of an accentuated Abietinae-type, scarcely a trace of it being present in the apical region of the prothallium. The coat is double in the basal region in two of the forms about the time of fertilization, and will probably be found to be similar at a like stage in *Gnetum*. Material of this form was not examined, however, and the point remains in doubt.

FOSSIL GYMNOSPERMOUS SEEDS.

Numerous seeds of a primitive gymnospermous character occur in the palaeozoic rocks. Many of these with the structure well preserved are found in the Carboniferous and Permian strata. Hooker and Binney[15] were the first to study their internal features. Later Brongniart[31] and Williamson[16] worked out in detail their intimate structure. A glance over the illustrations of these investigators gives one an idea of the prominence of the megaspore-membrane in the early seed-plants. Brongniart whose specimens were exceptionally well preserved refers to the megaspore-coat as follows, (Brongniart,

31, p. 242) : "La membrane intérieure ou périspérmique est très différente de celle qui limite le nucelle ; elle est extrêmement mince et ne paraît pas cellulaire, mais marquée d'aréoles dûes à l'application des cellules qu'elle enveloppait et dont il ne reste généralement plus de trace." Williamson's specimens were not in so good a state of preservation but the megaspore-membrane is represented in many of the figures in his paper. In the text also he often refers to the coat, and compares it with the similar structure described by Brongniart. These Palaeozoic seeds belong to a great variety of forms, some related to the cycads, some to the conifers, and some, probably very many, to the Cordaitales, the dominant gymnosperm group of this era. Others again are seed-bearing Cycadofilices, the Pteridospermae, a group recently established by Oliver and Scott.[7] Still others are lycopods with seed-like fructifications which Dr. Scott has described under the generic name of *Lepidocarpon*. The latter have an especially prominent megaspore coat, which in some cases is plainly double as its separation into two layers in the chalazal region indicates (Scott,[28] figs. 27 and 28).

In the mesozoic rocks the abundant remains of gymnospermous plants belong chiefly to the Bennettitales, a group with strong cycadean affinities. Carruthers[32] in 1870 described for the first time the female strobilus in *Bennettites*, the type genus of this group. Neither he, Solms-Laubach, nor Scott, who have studied the European forms, have observed that a megaspore-coat is present. In fact, for the only structure which might be interpreted as such Solms-Laubach distinctly claims a nucellar origin (Solms-Laubach,[33] pp. 441 and 442). The American representatives of the Bennettitales are being worked over by Dr. Wieland who has already made very important additions to our knowledge of the reproductive organs of the group. In correspondence in regard to the presence of a megaspore-membrane Dr. Wieland states that he is unable to affirm directly that such a structure is present in the material he has examined. With regard to other mesozoic gymnosperms, the forms that are related to the modern ones, the

ancestral Ginkgoales, Coniferales, etc. I have been unable to get any information on the presence of a megaspore-membrane that is of value.

It is thus evident that in these fossil seeds the more primitive, palaeozoic forms have a much more prominent megaspore-coat than the specialized and more recent mesozoic ones, in which even the occurrence of a coat does not seem to have been demonstrated with certainty.

GENERAL CONSIDERATIONS.

The present work has determined the extent of the occurrence of a megaspore-membrane in the gymnosperms, as well as the structure of the coat and its chemical composition. The megaspore membrane is present in all the groups and subgroups of these seed-plants, except the Taxeae of the Coniferales, from the ovule of whose forms it is entirely or almost entirely eliminated. The coat is strikingly uniform in structure and in chemical composition throughout this division of the spermatophytes with the exception of one subgroup, the Araucariinae. It is double, its exosporium, in the later stages of development, composed of radially arranged fibrillae, and its endosporium presenting an appearance, in section, of being subdivided into two more or less equal, homogeneous strata. The exosporium is suberized while the endosporium is of composite chemical character. The outer stratum of the latter is suberized but contains cellulose towards its inner border, while the inner stratum consists chiefly of cellulose with which entad is associated, a substance resembling pectin. The megaspore-coat in fact closely resembles that of a microspore (e.g. that of *Pinus*) both in its structure and in its chemical composition, and thus affords additional evidence of the free-sporing nature of the ancestral forms of the gymnosperms.

In the forms where the normal type of membrane occurs there is present a more or less well-developed tapetum. This tapetum is derived from the sporogenous tissue as is shown by the course of its development, the plurinucleate condition of its cells, and by the suberization of their walls. It is quite

distinct from that which is derived from the nucellar tissues and has for convenience been designated a "primary" tapetum.

The abnormal type of megaspore-membrane present in the Araucariinae is comparable to the wall of the pollen-tube both in structure and in chemical composition, a typical suberized exosporium not being present. This group in many other respects occupies a somewhat isolated position among the subgroups of the Coniferales. The tapetum is of a peculiar character in both *Agathis* and *Araucaria*,—just as abnormal as is the megaspore-membrane. The "female flower," too, is difficult to homologize with that of any of the other forms. Some consider that the seminiferous scale is a very reduced structure being composed of the almost completely fused fertile and infertile bracts, while others regard the ovule-bearing structure as a simple sporophyll, and on this ground consider the Araucariinae as the most primitive of conifers. In support of this view reference is often made to the evidence afforded by early occurrence of fossil Araucaria-like wood. Dr. Scott[34] (p. 483), however, finds the case in this respect "emphatically ‚not proven' on existing evidence". The character of the coat and of the tapetum is in keeping with his finding and indicates in addition that the Araucariinae are to be regarded as a specialized subgroup of the Coniferales.

Leaving out of consideration the Araucariinae whose megaspore-membrane and tapetum cannot at present be satisfactorily associated with the other gymnosperms, certain general features which are important from the phylogenetic standpoint have been demonstrated. The coat is thick, well developed, and of fairly uniform distribution around the prothallium in the Cycadales, the group which is recognized as the most primitive of the modern gymnosperms. In the Ginkgoales it is thinner than in the Cycadales but similar in distribution to that of the latter. The group is a recently established one for the reception of the single form *Ginkgo biloba*, which was previously included in the Taxeae of the Coniferales but which is now considered "as the one surviving

member of an ancient stock, derived from the same cycle of affinities as the palaeozoic Cordaiteae" (Scott,[34] p. 485), and has been given a phylogenetic position below the Coniferales. In the last mentioned group, which comprises the much diversified forms of the present-day gymnosperms, the coat is very varied in thickness and peculiar in its distribution around the prothallium. It is, however, though thinner than in *Ginkgo*, on the whole much thicker and more fully developed than in the Gnetales, the group of gymnosperms which is recognized as having the greatest affinity to the angiosperms. Thus in the living forms it is seen that there is a direct relationship between the thickness and state of development of the megaspore-coat, and the primitive character of the group, a progressive destruction of the coat having gone on as the forms become more specialized. The development of the suberized primary tapetum in the different groups parallels that of the megaspore-coat and affords confirmation of the evidence derived from the state of development of the latter. Again in the fossil forms the primitive palaeozoic representatives have a much thicker megaspore-coat than the higher and more specialized mesozoic ones, since, as was seen, the coat in the former is described as a well preserved structure, while in the latter it is so poorly developed as to have escaped observation, or, at least, description. Thus in both the great modern and fossil groups of primitive seed-plants we have evidence that the megaspore-coat varies in thickness according to the primitive or specialized nature of the forms. That this is true of the subgroups as well as of the large divisions is indicated by the study of the megaspore-membrane of the Cycadales, the Cycadeae having the thickest and the Euzamieae the thinnest coat.

The interpretation of the inter-relationships of the subgroups of the Coniferales in the light of the above generalization is the chief object of the present work, and in connection with the statement of the results obtained reference will be made to certain other features of general phylogenetic importance which are in keeping with the evidence afforded by the state of development of the megaspore-coat.

All the subgroups of the Pinoideae have a megaspore-membrane and a tapetum. Those of the Araucariinae are of a specialized nature and have been referred to separately. Of the other subgroups of the Pinoideae, the Taxodinae, which Eichler in his classification has placed between the Abietinae and the Cupressineae, show affinity to each of these subgroups in the character of the megaspore-coat and the tapetum. Reference has already been made to Arnoldi's proposed separation of the two species of the Sequoias (p. 37). In the same paper he gives it as his opinion that all the Taxodinae (Sequoiaceae) except *Sciadopitys* would be better associated with the Cupressineae. The evidence afforded by the study of the megaspore-coat of these forms is in favour of Arnoldi's view and indicates that the Taxodinae is a composite group, all the forms examined, except *Sciadopitys*, being best associated with the Cupressineae to which their megaspore-coat approximates in its state of development. The associated forms of the Taxodinae and the Cupressineae have a very thin membrane and a poorly developed tapetum, being in this respect highly specialized, the true Cupressineae on the whole somewhat more so than the Taxodinae. The forms of the combined groups resemble one another in certain other features, such as the absence of male prothallial cells, the grouped arrangement of the archegonia and the degenerate nature of the brachyblast, all which point to their specialized character. The cyclic arrangement of the leaves and sporophylls in the Cupressineae proper is evidence of a similar nature.

The megaspore-coat of *Sciadopitys* is in distribution and in structure similar to that of the Abietinae at the same stage of prothallial development (see p. 34). It is, however, fully one-half thicker, being almost or quite as thick as the coat of *Cycas* at a similar stage. The tapetum of *Sciadopitys*, too, is thick, especially so in the basal region, and its distribution thus suggests the state of affairs at a later stage in the Abietinae, when the tapetum consists of only a few cells in the basal region. The megaspore-coat and the tapetum of *Sciadopitys* would thus seem to be of a primitive abietineous type.

Other features as well indicate its affinity to the Abietinae. Arnoldi has referred to the distribution of the archegonia as being similar to that in the Abietinae. Again, in the vegetative parts the development of long shoots and short shoots is characteristic of both. The so-called "leaves" of *Sciadopitys* are peculiar and lend confirmation to the brachyblastic theory of the seminiferous scale, which upon anatomical grounds is considered to hold good for all the Coniferales. This interpretation of the character of the ovule-bearing structure of the Coniferales gains support from teratological evidence, and is "in favour of the view that the Abietinae, and the Taxodineae as well, are somewhat primitive orders" (Jeffrey[35], p. 456) since in these subgroups alone do proliferous cones occur. In this connection attention is directed to the very common occurrence of proliferating female cones in *Sciadopitys*, (see Sir W. T. Thiselton-Dyer's reference to Master's work, *Ann. Bot.*, 1903, pp. 779-787), and to the additional evidence which is thus afforded of the primitive nature of this form. The presence of two prothallial cells in the microspore, and the lack of differentiation in the male cells themselves, only one of which functions in fertilization, are features which point in the same direction and corroborate the testimony afforded by the state of development of the megaspore-coat and the tapetum.

The Taxoideae have one order, the Taxeae, which must be regarded as very specialized from the standpoint of the development of the megaspore-coat and the primary tapetum, since no such structures, or only traces of them, are present in the three of its genera examined. In keeping with this condition of affairs the female "flower" is of a very specialized type. The axillary buds (brachyblasts) are reduced in number and degenerate in organization. Moreover, no prothallial cells are present in the microspore, and the single functional male cell (in *Taxus* at least) is relatively very large and specialized. In addition *Taxus* is the only one of the Coniferales in which no resin ducts are developed, a feature in which it resembles the Gnetales. Again, in *Torreya* a *secondary, nucellar*, but no

primary tapetum is developed. In the other suborder of the Taxoideae, the *Podocarpeae*, there is a great difference in the stage of development of the megaspore-coat and the tapetum in its two chief genera. Reference (see p. 43) has already been made to certain associated differences which are in keeping with the former and from which it would appear that *Dacrydium* is a much more primitive genus than *Podocarpus*. Certain resemblances of the whole group to the Abietinae have been recently pointed out by Dr. Coker, such as the occurrence of winged pollen grains, the arrangement of the archegonia, the presence of two prothallial cells in the microspore (*Podocarpus* and perhaps others) and certain other features which have led him to conclude "that in the Podocarpeae are to be found the nearest living relatives of the Abieteae" (Coker,[26] p. 103).

From the standpoint, then, of the relative state of development of the megaspore-coat and the tapetum we are to regard the Abietinae as the most ancient group of the Coniferales; the Taxeae as the most recent; the Taxodineae and the Podocarpeae as complex groups, with some forms as ancient as, or even more ancient than the Abietinae, and other forms quite recent,—while the Cupressineae are considered as occupying a somewhat intermediate position in the phylogenetic series.

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EXPLANATION OF PLATES.

Plates 1 and 2 consist of drawings which were outlined by the aid of a camera lucida. The drawings were made from the lateral basal region of the prothallium except where otherwise stated, since the membrane in this region is more uniform and assumes about its average thickness. The figures are oriented so that the prothallial tissue is to the extreme left of each. An attempt has been made both by the use of the camera lucida and by actual measurement to reproduce the relative thickness of the coats in the various forms. For accurate comparison however, the measurements must be taken as the basis. Only in a few cases has any attempt been made to indicate the stratification of the endosporium.

Plates 3-5 are reproductions of photomicrographs, and for details of structure should be examined with a lens.

Plate 1.

Fig. 1. *Cycas revoluta*. (4. 5 μ) The endosporium and the exosporium are about equal in thickness. The former presents the appearance of being subdivided by a broad and coarsely granular area ; the latter is finely striated. The tapetal cells are large and contain many amylodextrous starch grains. These appear in the unstained condition as vacuoles.

Fig. 2. *Ginkgo biloba*. (4.5-5 μ) Membrane of adult seed, separated slightly from the endosperm cells, the outer walls of which are especially thick and have an outer suberized layer. The exosporium is relatively very thick and is finely and irregularly striated. The tapetal and nucellar tissues consist of collapsed strands.

Fig. 3. *Pinus resinosa*. (4.2 μ) For the stage see photo. 4, pl. 3. The endosporium is about one-third as thick as the exosporium. There is but a slight "reinforcement" from the endosperm cell walls. To the right of the membrane the tapetal tissue is represented and the inner edge of the nucellus as well.

Fig. 4. *Larix Europaea.* (4.4 μ) The walls of the endosperm cells are just forming. The tapetum consists of a single layer of rather collapsed cells.

Fig. 5. *Larix Americana.* (4.7 μ) The drawing is from the basal part of the megaspore. The membrane is like that of *L. Europaea*, but of heavier structure and slightly "reinforced" by the outer walls of the endosperm-cells. The tapetal cells are large in this region.

Fig. 6. *Picea excelsa.* (4.7 μ) The stage of development is indicated in photo. 7, pl. 3. The exoporium is relatively very thick. It is finely and very regularly striated in structure. The reinforcement is slight.

Fig. 7. *Picea nigra.* (4.6 μ) Basal region of megaspore-membrane- very similar to that of *P. excelsa.* Collapsed tapetal cells are present.

Fig. 8. *Tsuga Canadensis.* (4.5 μ) Membrane as in last two but thinner. To the right of the exosporium is a layer of tapetal débris which is really closer to the membrane than it has been represented and makes the membrane appear quite thick (See photo. 8, pl. 4).

Plate 2.

Fig. 9. *Abies balsamea.* (4.6 μ) The stage of the ovule from which drawing was made is seen in photo. 9. pl. 4. The membrane is slightly "reinforced." The endosporium is homogeneous and about one-fourth as thick as the exosporium which is very distinctly and coarsely striated.

Fig. 10. *Pinus sylvestris.* (3 μ) Membrane of an ovule at a slightly earlier stage than that indicated in photo. 10, pl. 4, in the parietal nucleated condition. The endoporium is homogeneous while the exosporium is finely granular and shows signs of an indistinct radial striation. To the right of the megaspore the tapetal cells appear as quite a distinct layer.

Fig. 11. *Sciadopitys verticillata.* (4.2 μ) The stage of the ovule is indicated in photo 11, pl. 4. Cell formation is proceeding in the megaspore. The exosporium is very finely granular and radially striated and about one and one-half times as thick as the endosporium, whose two substrata are fairly distinct.

Fig. 12. *Sequoia sempervirens.* (2-7 μ). The drawing was made from a part of an ovule (at about the stage indicated in photo. 12, pl. 4) where the membrane was free from the nucellar tissues. Usually it is more closely pressed up against the latter than the figure would indicate, no tapetum occuring between them.

Fig. 13. *Biota orientalis.* (1.7 μ) The drawing shows that the apparently thick megaspore coat indicated in photo. 14, pl. 5 is not really a true megaspore-membrane but that a great part of the thickness of the investing layer is made up of the thickened outer walls of the peripheral endosperm cells. The endosporium is homogeneous, the exosporium granular.

Fig. 14. *Juniperus Sabina.* (2.7 μ) The stage of development of the ovule is indicated in photo. 16, pl. 5. The membrane seems to vary somewhat in thickness in different parts of the section. The two layers of the coat are about equally thick. The endosperm cells "reinforce" the membrane considerably. To the right the collapsed tapetal cells are evident and the inner border of the nucellar tissues.

Fig. 15. *Dacrydium laxifolium.* (4.3 μ) Membrane at the stage indicated in photo. 17, pl. 5. It invests closely the endosperm and is "reinforced" by the outer wall of the peripheral endosperm cells, the thickness of which is about equal to that of the inner layer of the membrane proper. The exosporium is coarsely and irregularly striated. The tapetal tissue has collapsed.

Fig 16. *Welwitschia mirabilis.* (1.3 μ) At the stage indicated in photo. 19, pl. 5, the megaspore-coat is thin even in the lateral basal part of the ovule. The inner border of the nucellar tissue consists of very much collapsed cells.

Plate 3.

Photo. 1. *Cycas revoluta.* (Meg-memb. 4.5 μ thick.) The ovule is at a stage just previous to cell-formation in the prothallium. The coat is double, its two layers about equally thick. (For details see fig. 1, pl. 1.) An artificial separation of the layers is indicated about the middle of the photograph. The staining is such as to bring out the layering of the coat but not the structure of the prothallial and other tissues.

Photo. 2. *Stangeria paradoxa.* (Meg-memb. 4.2 μ thick.) The endosporium appears subdivided in this case. Overstaining for contrast effect has obscured the structural details of the layers. The large cells of the tapetum are indicated to the right and the gametophyte to the left.

Photo. 3. *Araucaria imbricata.* (x 50) Archegonial initials have developed in the lower lateral parts of the prothallium. The distribution of the tapteum is fairly well indicated, though usually much more is seen in the apical region.

Photo. 4. *Pinus resinosa.* (x 20) The megaspore-membrane closely invests the prothallium, becoming much thinner in the archegonial region. The archegonium has not been fertilized. Remains of the tapetum are apparent in the basal region.

Photo. 5. *Larix Europaea.* (x 25) Several archegonia are present, two with neck and ventral canal cells. The megaspore-membrane is scarcely perceptible in the archegonial region and very thin for some distance below this, while in the chalazal region it is thick. The basal portion of the tapetum and of the nucellus have been torn away.

Photo. 6. *Picea nigra*. (x 50) Just prior to fertilization. Megaspore membrane somewhat broken but closely investing the prothallium while the tapetal remains are sparse but distinct in the basal region.

Photo. 7. *Picea excelsa*. (x 25) Some of the archegonia have been fertilized and the first nuclear divisions have taken place. Others have not been fertilized. The megaspore-membrane thins out uniformly towards the archegonial region. The tapetum is apparent in the basal region.

Plate 4.

Photo. 8. *Tsuga Canadensis*. (x 40) The megaspore coat thins out somewhat in the archegonial region, but not so much as in *Larix*, etc. The tapetum is pretty well destroyed but can be detected in the basal region. Granular material adds somewhat to the apparent thickness of the coat.

Photo. 9. *Abies balsamea*. (x 20) The ovule has been fertilized and the megaspore-coat is very thick in the basal region thinning out gradually towards the micropylar part. Tapetal remains are evident in the basal region.

Photo. 10. *Pinus sylvestris*. (x 80) Ovule in longitudinal axial section. The coat is double and evenly distributed around the prothallium, which is loosely invested by tapetal cells.

Photo. 11. *Sciadopitys verticillata*. (x 40) "Revived" material. Longitudinal axial section of an ovule at the stage when archegonial initials are appearing. The megaspore-membrane is of uniform thickness all around the prothallium. The tapetum is thick, especially in in the basal region.

Photo. 12. *Sequoia sempervirens*. (x 50). The megaspore-membrane is attached more or less to the nucellar tissue. The endosperm is becoming cellular and collected towards the chalazal region. The section passes through several megaspores towards the micropylar end.

Photo. 13. *Cryptomeria Japonica.* (x 100) No membrane is to be seen at this stage. The tapetum consists of a single rather loose layer of cells.

Plate 5.

Photo. 14. *Biota orientalis.* (x 10) Apparently shows quite a thick megaspore-membrane at the stage when numerous rudimentary embryos are developing. The explanation is given in fig. 13, pl. 2, where the "reinforcement" from the cell walls is seen to be very thick.

Photo. 15. *Thuja occidentalis.* (x 20) A young embryo is seen in the upper portion of the prothallium. The megaspore-membrane is thin even in the basal region, and the tapetum not apparent.

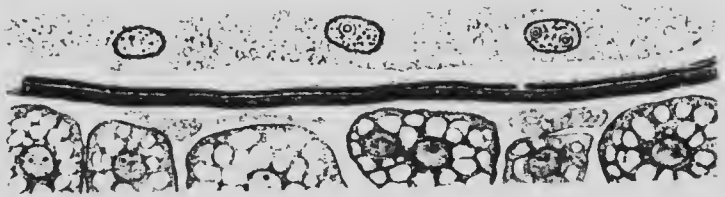
Photo. 16. *Juniperus Sabina.* (x 20) The megaspore-coat appears very thick, much thicker than it really is since it is "dragged". The tapetum can be seen in the basal region. In the archegonial region the contents of several pollen-tubes are apparent.

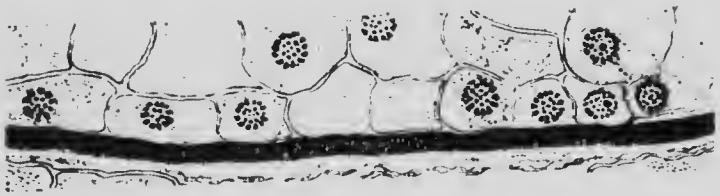
Photo. 17. *Dacrydium laxifolium.* (x 20) Sections of dry herbarium material "revived" 4 years after collection. The integument and nucellus have been removed. The megaspore-membrane is thus the thick coat enclosing the prothallium in whose axis embryonic and suspensorial cells are visible.

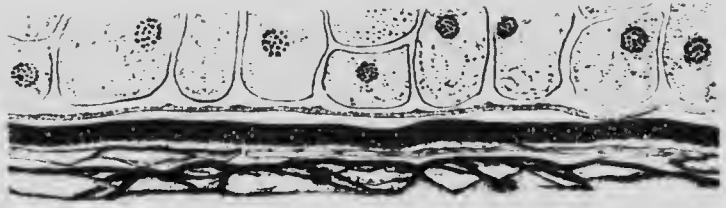
Photo. 18. *Taxus Canadensis.* (x 50) The nucellus and contained structures only are present. Above the embryonic and suspensorial cells a pollen-tube without any contents is apparent.

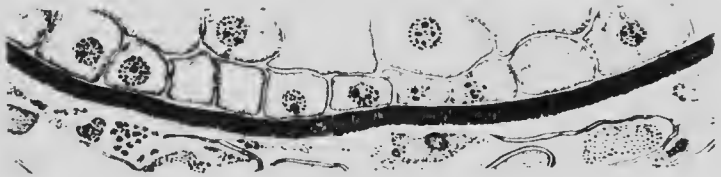
Photo. 19. *Welwitschia mirabilis.* (x 25) The megaspore-membrane lies free, midway between the endosperm and the nucellus. It thins out very perceptibly towards the micropyle. Suspensorial cells with a triangular mass of embryonic tissue at the base are visible in the longitudinal axis of the prothallium about one-third of the distance from its apex.

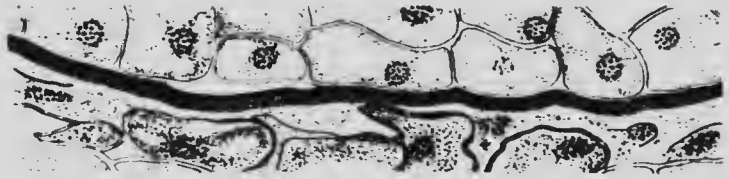
Photo. 20. *Ephedra distachya*. (x 12) The megaspore-membrane is fused with a felt-work from the adjoining nucellar issue. Numerous free nuclei are present in the egg-cells.

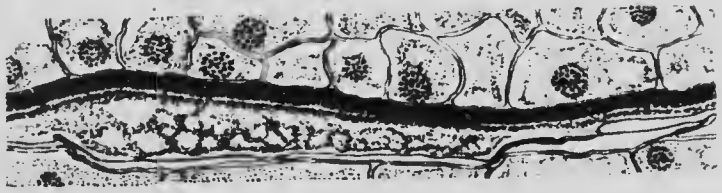


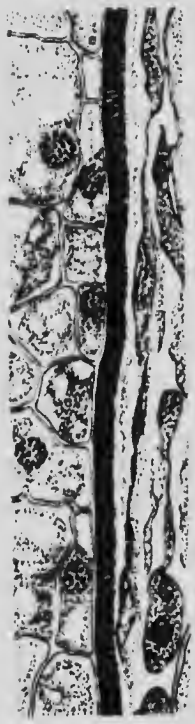












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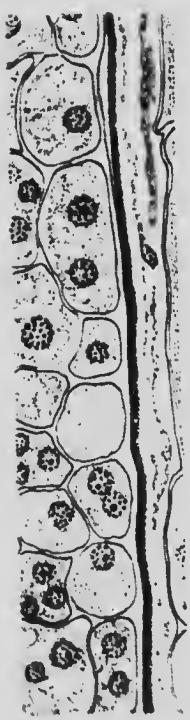

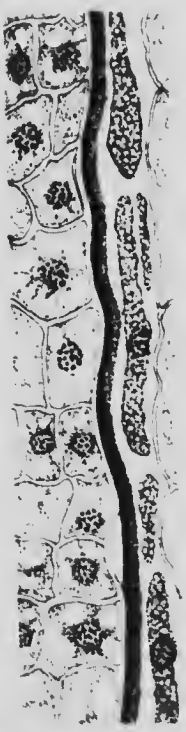

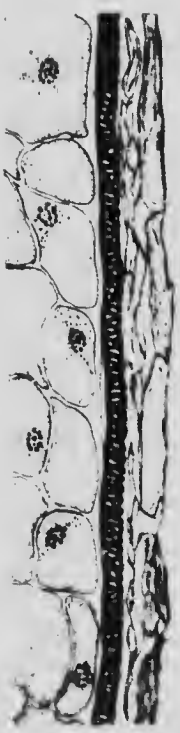

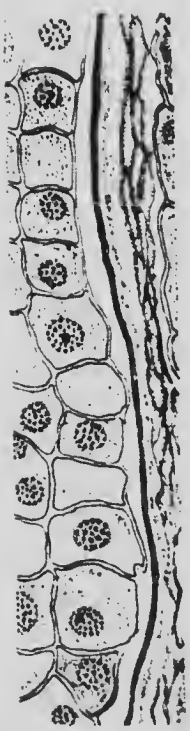

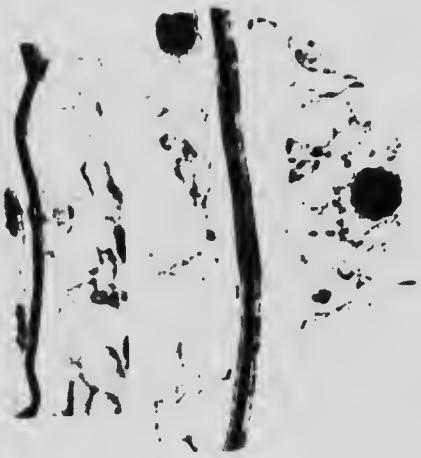

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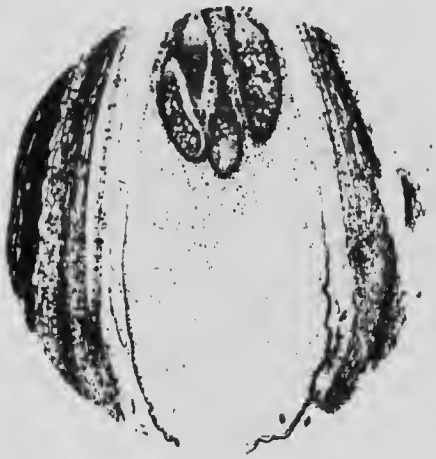

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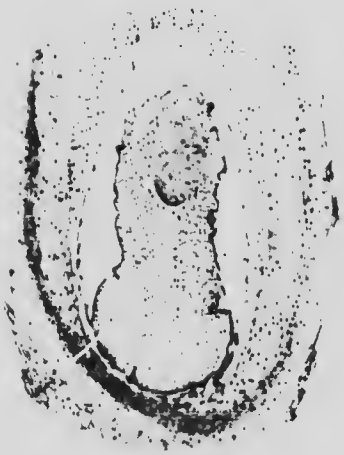

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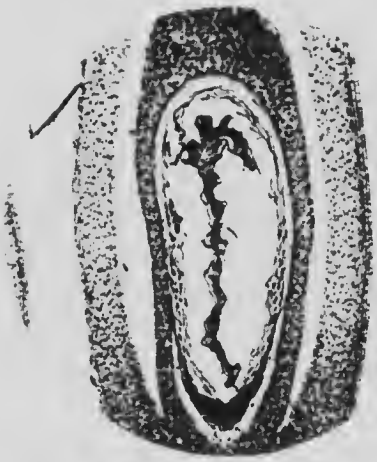

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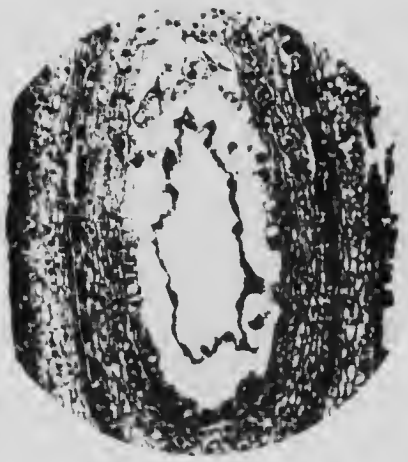

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