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Studies in Spore Development.

BY

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With Plate XIII.

Introduction.

DURING the eighties of last century great activity was displayed in the investigation of the structure and development of the spores and pollen-grains of a large number of plants. The first impetus to this activity was due to the splendid investigations of Strasburger upon this subject, which he published in his treatise 'Ueber den Bau und das Wachsthum der Zellhäute' in 1882 (16). Two years later Hubert Leitgeb issued his studies upon the structure and development of a number of spores with particular reference to those of the Hepaticae. In 1886 appeared Wille's 'Ueber die Entwickelungsgeschichte der Pollenkörner der Angiospermen', whilst in 1889 Strasburger published a further series of studies on spores and pollen-grains in Heft II of his 'Histologische Beiträge'.

After this date there was a temporary lull in the investigation of spore histology, and until the opening year of the new century the only important contribution to the subject—apart from the interesting microchemical work of Mangin—is yet another publication of Strasburger's in 1898 upon 'Die pflanzlichen Zellhäute' (18).

In 1900 an important account of the structure and development of the spores of *Isoëtes* and *Selaginella* appeared from the pen of Hans Fitting (5). In this work, crowded with a wealth of interesting details, probably the most unexpected feature was the demonstration that the spore-walls could carry on their growth although the protoplast of the spores was not in contact with them during this process. Fitting's discovery was very soon confirmed in *Selaginella* by Campbell (2), and a little later by Denke (4). Miss Lyon (10 *a*) has given a different interpretation of the growth of these membranes, but I think her objections have been sufficiently met by Fitting's reply in the 'Botanische Zeitung' (6). In 1905 I was able to find another case (*Oenothera*) in which the pollen-membranes possess independent powers of growth

[Annals of Botany, Vol. XXV. No. XCVII. January, 1911]

whilst the protoplast is not in contact with them. In 1908 Tischler (21) observed another and most striking example of this phenomenon. In the pollen-grains of *Mirabilis Jalapa* the protoplasmic contents degenerate and shrink to a scarcely noticeable quantity, and yet notwithstanding this the exine of these grains continues to grow very considerably in thickness and extent. For some time past I have been examining a large number of spores and pollen-grains belonging to many species of plants in order to find, if possible, other examples of membranes which are able to continue their growth without the direct co-operation of the protoplasm. I have, up the present, found no other such striking cases as those of *Selaginella*, *Isoëtes*, *Oenothera*, or *Mirabilis*, but a careful examination of so large a number of different spores could not fail to bring to light many interesting details which supplement our present knowledge of the subject.

I have already given a description in these pages[1] of two of these spores (viz. *Helminthostachys* and *Riccia*), and I now propose to add an account of some other pollen-grains and spores which I have had under observation. I intend to deal with the pollen-grains of *Ipomoea* in the present part.

I. Ipomoea.

The only account of the finer structure of the pollen-grains of a species of *Ipomoea* is the very short description given by Strasburger in 1889 (17). After having furnished a minute account of the spinous pollen of several species of Malvaceae, Strasburger devotes a few lines to the pollen-grains of *Ipomoea coccinea*, Moench., which appeared to him to be constructed quite after the manner of the Malvaceous type.

I have examined the pollen-grains of *Ipomoea purpurea*, Roth.,[2] in some detail, and as I find that these differ in several respects from the Malvaceous type of pollen, I will begin these 'Studies' with a description of them.

My material was fixed partly in Flemming's solutions and partly in chrom-acetic mixture without osmic acid. So far as possible I have checked my results by comparison with living material examined in 0·6 % NaCl solution, but the opacity of the structures did not render this method a very satisfactory one in the present case.

The pollen mother-cells of *Ipomoea* usually form two or sometimes three longitudinal rows in each pollen-sac; they are each surrounded by a wall which gives the reactions of callose and also of pectose, and they include a rather large nucleus (about 14 μ in diameter) which contains, as a rule, a single large nucleolus and a loose reticulum of fibres. The tapetal cells,

[1] Ann. of Bot., vol. xx, April, 1906, pp. 177–186, and Ann. of Bot., vol. xx, July, 1906, pp. 275–291.

[2] This plant is also known as *Pharbitis hispida* (Choisy).

which form a single layer round the mother-cells, are radially elongated structures which in the majority of cases enclose two nuclei, although cells with three or even four nuclei are met with. The stages of the division of the pollen mother-cells were but poorly fixed in my preparations, and, with the exception of the telophase of the second meiotic division, which was well shown in my sections, I will make no reference to the subject.

At the conclusion of the second division of the pollen mother-cells the chromosomes retain their individuality for some time after a nuclear wall has been reconstructed and a new nucleolus (or nucleoli) formed in each daughter nucleus (Pl. XIII, Fig. 1 *a*). The chromosomes are distributed throughout the nuclear cavity, but are connected with one another by a delicate linin threadwork. A little later the sharply defined, homogeneous chromosomes become more irregular in outline and apparently vacuolar in structure (Fig. 1 *b*), and it is easy to trace the gradual opening out of their substance and its distribution over the linin network until no trace of any individual chromosome can any longer be detected (Fig. 1 *c*). After the division of these nuclei is finally completed, therefore, nothing can be seen in the nature of the 'prochromosomes' which Rosenberg (14, 15), Overton (12, 13), Laibach (10), and others have described in the 'resting' nuclei of various plants.

I have examined the nuclei of the other tissues of the anther for prochromosomes, and in some, notably those of the young vascular tissue, chromatic aggregates are to be seen lying beneath the nuclear wall which resemble in appearance the prochromosomes of other writers (Fig. 1 *d*). Their number, however, appears to me to be too inconstant in these cells to have the significance which attaches to true prochromosomes.

There is evidently great variation in the behaviour of the chromosomes of different cells at the conclusion of nuclear division. In some, such as in the cases described by Rosenberg and others, the chromosomes appear to retain a large proportion of their material definitely aggregated as clearly distinguishable prochromosomes throughout a prolonged period of rest. In other cases, such as the pollen mother-cells of *Ipomoea*, the chromosomes retain their individuality as distinct bodies for a short, but yet quite definite, period, and then their substance becomes evenly distributed over the linin reticulum. Finally, in many other cells the chromosomes become vacuolated and their substance dispersed over the linin threadwork immediately at the conclusion of mitosis. In cells such as those of the young vascular tissue of *Ipomoea*, in which an inconstant number of chromatic aggregates occurs, it is not improbable that some of the chromosomes may remain visible as distinct bodies for a greater or less time, others may become distributed over the linin at once and lose their visible individuality, whilst others may become vacuolated and broken up into two or more smaller but still recognizable bodies.

The tetrads of young pollen-grains become surrounded by massive mucilaginous walls. Upon the periphery of each tetrad group is a granular deposit which stains with Bismarck brown but not with aniline blue or corallin soda. This is probably the remains of the primary wall which separates the sporogenous cells from one another. Within this a distinct, often rather massive, layer is seen which possesses the staining properties characteristic of Mangin's callose and of pectose.[1] This is the mother-cell wall already referred to above. Within this again is another mucilaginous wall which also gives the reactions of callose and pectose and which envelops the young pollen-grains and separates them from one another. For this innermost wall Strasburger (19) has recently suggested the convenient name of *Special wall* to replace the old term Special mother-cell wall with its false implication (Fig. 2).

In microtome sections which have been stained either with aniline blue or Congo red three radiating lines (really lamellae), often of a granular appearance, can be seen to traverse the middle of the special wall (Fig. 2). These granular bands are the first lamellae which are formed at the conclusion of the division of the mother-cell. In my sections stained with Heidenhain's iron-alum haematoxylin and Bismarck brown these lamellae are often quite unstained and appear as colourless clefts or lines in the middle of the brown special wall. Mangin (11) has described similar triradiate lines of granules in the case of *Althaea rosea*, and he states that they are nitrogenous in character. I was unable to determine their chemical nature in the case of *Ipomoea*.

At the time when the callose-pectose walls break down and set the pollen-grains at liberty it is often seen that the triradiate lamellae continue to exist for some time in the midst of the flocculent material derived from the rest of the wall (Fig. 3).

The callose-pectose layers of the special wall which immediately envelop the pollen-grains and which are the latest parts of this wall to be formed are denser than those in the neighbourhood of the granular lines.

There is evidence that the special wall of *Ipomoea* possesses a laminated constitution.

The young pollen-grains of *Ipomoea* surround themselves with a wall of their own—the exine. This is deposited by the pollen-protoplast as an extremely delicate layer upon the inner face of the callose-pectose wall which surrounds it. From the first it is marked off as an independent structure from the callose-pectose special wall, and there can be no doubt that it is a new membrane and not one derived from the transformation of the innermost lamellae of the special wall.

In its earliest stages it is an exceedingly delicate membrane, which is too thin to permit any structure to be seen in it even with the highest

[1] Compare Tischler (21), p. 48.

powers of the microscope and in the most delicate microtome sections. In Fig. 4 a young pollen-grain is represented lying within the special wall. Here the pollen-protoplast has contracted under the influence of the re-agents, and the young exine has also separated from the special wall from the same cause. Under these circumstances the newly developed exine can be seen exceptionally well as an independent membrane of great tenuity. In somewhat older pollen-grains a structural differentiation of the exine can be detected which even at this early stage exhibits some complexity. The exine can now be seen to consist of an outer lamella, upon the inner face of which is deposited a network of thickening bands. At the angles of the meshes of this network the rudiments of the future spines already occur. Between the thickening bands and the outer lamella a narrow cleft or unstained space can be seen, and this is the position in which the rodlets of older pollen-grains are developed.

Fig. 5 shows the inner face of the exine at this stage in surface view. The more deeply staining system of thickening bands is seen to form a reticulum with polygonal (mostly hexagonal) meshes upon the lighter outer layer of the exine. At the angles of the network the spine rudiments are seen as deeply coloured dots.

Fig. 6 *c* represents the same stage in section. Here the alternation of thicker areas, where the thickening bands lie, with thinner intervals is seen. In very delicate microtome sections the separation of the thickening band from the outer lamella of the exine by a clear, unstained space or layer can readily be made out (Fig. 6 *b*).

Where the section has passed through the spine rudiments the appearance is somewhat different. In Fig. 6 *a* it will be seen that the thickening band appears to be pushed inwards (by the colourless layer) at each spine rudiment so as to form an internal spine. The spine rudiment itself appears as a deeply stained particle just within the apex of each of these projections. The external surface of the exine is still completely flat and smooth.

The mode of development which these component parts of the exine follow is a difficult matter to decide with certainty. There can be little doubt, I think, that the outermost lamella and the system of thickening bands are successive developments secreted by the pollen-protoplast one after the other. The thin structureless membrane of such stages as that represented in Fig. 4 I believe to correspond to the outermost lamella alone. Upon this the bands of thickening are laid down by the protoplast in somewhat older pollen-grains. At first these bands are so thin and faintly marked as to appear as little more than shadowy traces upon the inner face of the membrane, but they rapidly gain in distinctness as development proceeds and new material is added to them by the protoplast. Exactly how and when the spine rudiments and the rodlets are first developed is a more difficult problem to determine. The impression which I have

gained from the study of my preparations is that the clear space which is seen in the sections to lie between the thickening bands and the outer lamella represents a third and distinct layer of substance (with little affinity for stains) deposited by the protoplast previous to the development of the thickening bands. This layer subsequently becomes differentiated into the spines and rodlets. This interpretation of the layers of the exine of *Ipomoea* would be more or less in accordance with the views expressed by Strasburger (18) in the cases of *Knautia* and *Althaea*, and by Tischler (21) for *Mirabilis Jalapa*.

It is, of course, possible that the layer containing the rodlets and spines may only become differentiated later, after the thickening bands have already been deposited, but I think this is unlikely both from the appearances in the present case and from analogy with what occurs in other plants.

The thickening bands quickly increase in both thickness and breadth as fresh material is added to them by the protoplast. During the early stages the substance of the thickening bands appears to be soft and mucilaginous; their outer margin is ill defined and encroaches upon the clear spaces or layers referred to above, so that these become difficult to distinguish, and the spine rudiments have the appearance of being embedded in the substance of the bands (Fig. 7).

The external surface of the pollen-grain still remains smooth as the spine rudiments do not yet project above its surface.

It may be noted that during the time that the various layers of the special wall and of the pollen-wall are being laid down by the protoplast, kinoplasmic fibres can clearly be distinguished running between the nuclear membranes and the 'Hautschicht' at the periphery of the protoplast (Figs. 4 *6 a* and 7). These fibrils can be traced back to the kinoplasmic radiations which surround the nuclei during the telophase of the second meiotic division. The persistence of fibrillar differentiations of this kind is by no means uncommon during the earlier stages of the development of the pollen-grain, and I have met with it in several other plants besides *Ipomoea*.

It seems quite probable that influences of some kind are distributed along these fibrils from the nucleus to the 'Hautschicht' which is taking an active part in the formation of the new cell-wall lamellae.

Up to the present the young pollen-grains have remained enclosed within the special wall. Now, however, these walls break down into a diffuse, flocculent material which fills the cavity of the anther loculus. The triradiate middle lamellae of the special walls, which we previously recognized as granular lines in the middle of the callose-pectose walls, often remain intact for some time longer, and can be seen lying in the midst of the flocculent material derived from the degeneration of the rest of these walls (Fig. 3).

Soon after this time the spine rudiments have grown sufficiently centrifugally to project very slightly above the outer surface of the pollen-grain, and to give this a wavy appearance. Before long they project far enough beyond the periphery of the exine to give this a distinctly spinous character. At about this period the 'rodlets' can first be clearly observed as minute deeply stained structures lying in the position of the clear space or layer noticed at an earlier stage between the outer lamellae of the exine and the thickening bands. Both the 'rodlets' and the spines now stain much more deeply than the rest of the exine, and they are, therefore, very clearly distinguished in the sections (Fig. 8).

At this time the kinoplasmic fibres running between the nuclear membrane and the 'Hautschicht' become obscure, and appear to merge into and become lost in the alveolar substance of the cytoplasm.

In rather older pollen-grains the relation of the parts of the exine to one another becomes much clearer. The thickening bands have increased greatly in thickness and have become much broader, so that the thin areas of the exine between these bands are now reduced to a series of pores or narrow channels which represent the exit pores for the future pollen-tubes. Moreover, the substance of the bands appears to have undergone a change, for these are no longer diffuse and ill defined at their inner margins, but they are now sharply marked off from the distinct 'rodlet' layer. The spines have grown greatly in size. They are still limited to the angles of the network of thickening bands, and they are now seen to be spindle-shaped with their points projecting for some distance beyond the still very delicate outer lamella of the exine and their 'roots' occupying the whole thickness of the rodlet layers. These 'roots', moreover, are seen to be double, each consisting of two prongs (Figs. 9 *a* and *b*). A surface view of the pollen-grains at this stage shows that the rodlets are limited to the positions overlying the hexagonally arranged thickening bands, and that they themselves, therefore, form a hexagonal figure when viewed from above. This is shown clearly in Fig. 18, although this represents an older pollen-grain in surface view.

The protoplast of the pollen-grain, which completely fills the pollen-cavity, has meanwhile become much poorer in substance and more vacuolated than at earlier stages. As the pollen-grain has increased from about 32 μ to about 45 μ in diameter the decrease in protoplasmic density is at any rate partly due to its substance being distributed over a larger area, but I believe that there is also a real loss of substance by the protoplasm, which has contributed some material to the growing membranes. The nucleus has only increased very slightly in size; the average of a number of measurements showed only an enlargement of about 2 μ (from 10 μ of an earlier stage to 12 μ now). The nuclear reticulum has become somewhat coarser and stains more deeply: one, two, or often more rather small nucleoli may occur.

The pollen-grain continues to increase in size, and its wall grows both in surface and in thickness; in proportion as this growth proceeds the protoplast continues to become more vacuolated and poorer in substance, although it never contracts away from the pollen-wall, as in *Oenothera*. In pollen-grains which measure 70 μ in diameter the cytoplasm encloses a number of large vacuoles, and the nucleus, which now measures about 14 μ in diameter, contains one or more nucleoli and a rather scanty reticulum. By the time the pollen-grains have reached 80 or 90 μ in diameter the cytoplasmic lamellae which separate the large vacuoles from one another have become broken down, and the protoplast is reduced to a hollow shell with a single huge vacuole occupying its entire centre. This cytoplasmic shell consists of little besides a 'Hautschicht', except in the immediate vicinity of the nucleus, where some granular cytoplasm still remains (Fig. 10). The nucleus is now a flattened body measuring about 20 μ by 10 μ across its greatest and least diameters. It enclosed a rather scanty, somewhat faintly stained arrangement of threads, and one, two, or more nucleoli of large size. The great increase of nucleolar matter is certainly the most striking change in the nucleus from its earlier stages; these large nucleoli may measure as much as 8 μ across. Not infrequently the interior of the nucleoli has a vacuolar appearance. The alteration in the appearance of the nucleus which is just beginning to become evident ushers in the process of protoplasmic reconstruction. The cytoplasmic shell is seen to become slightly thicker, and the granular cytoplasm, which had been reduced to one small area near the nucleus, can now again be observed as a thin layer all round the inner surface of the hollow protoplast. In the meanwhile, the nucleus has increased in size to as much as 30 μ by 20 μ in its longest and shortest diameters. The pollen-grain itself still measures 90 μ across. This nucleus enclosed one extremely large nucleolus (rarely two) which on an average measures about 12 μ in diameter. The fibrils which traverse the nuclear cavity have become much more numerous; they are finely granular in appearance and diffuse, and irregular in outline (Fig. 11). The protoplasmic shell continues to grow in thickness, and before long the single central vacuole becomes bridged across by one or two cytoplasmic lamellae which divide it up into a few large vacuoles. These become progressively smaller as the protoplasmic lamellae grow more massive and more numerous. Starch, which hitherto was present only in comparatively small quantities, now occurs in great abundance. Granules, or more probably droplets, and irregular masses of material which are black in my preparations stained with Heidenhain's haematoxylin also accumulate in the cytoplasm of the pollen-grain. The distribution of this dark-staining material in the pollen-protoplast is of some interest. It is usually rather densely collected in the little peripheral finger-like cytoplasmic processes which project into the exit pores of the pollen-wall. From these points the material can be seen

to spread out irregularly into the interior of the protoplast (Fig. 12). It has the appearance of a material, derived from without, which is making its entrance through the exit pores of the pollen-wall, which is then taken up by the little pseudo-podium-like processes of the protoplast, and which from these points becomes diffused through the cytoplasm of the pollen-grain. I have been able to obtain but little information with regard to the chemical nature of this substance. From the fact that it is blackened by the osmic acid in Flemming's solution it is probable that the material is of a fatty nature, but beyond this I can say nothing at present.

It may be noted that just about the time when this dark-coloured material is making its appearance in the pollen-protoplast, a number of vacuoles of varying sizes are formed in the tapetal cells, and that these vacuoles are filled with a material which is also darkened by osmic acid. I have not succeeded in tracing this darkened material out of the tapetal cells into the cavity of the anther and establishing a direct connexion with the similarly blackened substance in the pollen-protoplast, but such a relationship between the two appears quite likely. Moreover, the tapetal cells can be seen to undergo a loss in the total amount of substance they contain. These facts taken together suggest that the pollen-protoplasts are growing and storing reserve bodies in their substance at the expense of materials derived, at any rate in part, from the tapetal cells. During the earlier stages of the growth of the pollen-protoplast we find that its nucleus divides, and that the very unequal cell-division which follows cuts off a small generative cell from the large tube cell. The cytoplasm of the generative cell is almost entirely composed of kinoplasmic fibres radiating from its nucleus (Fig. 13). In older generative cells the fibrillar constitution of the cytoplasm gives place to a dense, almost hyaline structure.

A distinct plasma membrane limits the generative cell peripherally, but no cell-wall is developed (Fig. 14). The nucleus of the generative cell measures about 14 μ in diameter, and contains a comparatively large nucleolus and a rather loosely arranged system of fibres.

The tube nucleus is large, irregular, or even amoeboid in outline, and is distinguished by the enormous nucleolus and the system of deeply staining chromatic threads which it contains (Fig. 15). Amoeboid tube nuclei have been described in a number of other plants; for instance, in *Elodea canadensis* by Wylie (22).

The nucleolus of the tube nucleus is surrounded by a sheath of chromatin, and there are here no signs of the clear space (*heller Hof*) between the nucleolus and the chromatic reticulum of the nucleus which several writers have described (Fig. 15). In such cases as that represented in Fig. 16, where a slight contraction of the nucleolar substance has taken place at one spot, the relation between the nucleolar material and the chromatic sheath is particularly well seen. Martin Heidenhain, as long ago

as 1892 (and again in 1907) (9), described chromatic shells of this kind enveloping the nucleolus in several animal tissues, and his observation has been confirmed by other zoologists.

Cavara's (3) observations upon nucleoli may also be recalled in this connexion. This author described the nucleoli of higher plants as consisting of two parts: an external chromatic layer and an inner mass of plastin.

During this period of protoplasmic growth an intine has been formed on the inner surface of the pollen-wall. This layer is very thin over the general surface of the pollen-grain, but at each exit pore it is greatly thickened and protrudes towards the exterior. Where it is thickened the intine can clearly be seen to be composed of a number of lamellae, which suggests that its growth has taken place by the apposition of successive layers of material. An extension of the delicate outer layer of the exine covers the external surface of each protrusion of the intine at the exit pores of the pollen-grain (Fig. 17). The intine stains, although not very intensely, with those dyes which are characteristic of pectic bodies. Treated with calcium-chloride-iodine solution it gives at first no reaction, but after remaining in the solution for some days it is found to have coloured faintly violet. A preliminary boiling with dilute acid and alkali, according to Mangin's method, yielded no clearer cellulose reaction with the iodine reagents. From these reactions it may be concluded that the intine consists of pectic bodies associated with a little cellulose. The exine has meanwhile grown in thickness, and the relation of its parts to one another can now be very clearly seen.

The thickening bands of the exine have increased greatly in breadth and thickness, so that they now form a massive layer only perforated by the relatively small exit pores. This layer, which may be called the 'mesospore' according to Fitting's (7) terminology, possesses the reactions of a cuticularized structure (Fig. 17).

The outermost lamella of the exine (which we already saw at an early stage as an extremely delicate membrane) still remains very thin, and it can now be seen to possess an open structure perforated by countless little apertures which give it the appearance of a very fine reticulum in surface views. This perforated structure of the lamella is well seen in Fig. 18 and, in section, in Figs. 17 and 9. At the exit pores this reticulate layer dips down and covers over the protrusion of the intine. As at an earlier stage, we still find that the rodlets are limited to a hexagonal system of bands corresponding to the originally hexagonal disposition of the thickening bands (Fig. 18). At the angles of each hexagon is usually a spine. Both the spines and the 'heads' of the rodlets pass through the perforated outer lamella to reach the outer surface. The spines are spindle-shaped structures; their internal portions or roots are composed of two prongs, as already seen at an early stage. Fig. 18 shows that these

prongs have an hemispherical outline in transverse section. A stainable, homogeneous material lies between the rodlets under the reticulate outer lamella.

In sections which have been mounted in a drop of glycerine containing a little methylene blue and fuchsin mixture an interesting differentiation of the parts of the pollen-wall can be seen. The intine stains light red, the 'mesospore' is blue, the reticulate outer lamella of the exine, as well as the spines and the rodlets, is green, whilst the homogeneous sub-reticulate substance (between the rodlets) colours deeply red.

The mature spines of the exine measure between 12 and 14 μ in length; they are usually fusiform in outline, although I have occasionally found them with a dichotomously branched apex (Fig. 19).

The rodlets vary a good deal in size; their shape is usually like that of a drumstick with a part of the knob or head just projecting through the perforated outer lamella of the exine (Fig. 17).

The tapetal cells do not break down and scatter their contents between the pollen-grains, but they retain their membranes intact until the last. This tapetum, therefore, belongs to the 'secretion-tapeta' of Goebel.

Deeply staining fibres and granules occur in the cytoplasm of the tapetal cells of *Ipomoea* during the middle period of anther development. These are most probably similar to the chromidial structures which have been described in the tapeta of several other plants (*Nymphaea alba, Oenothera, Ribes, Lilium Martagon, Iris germanica, Syringa chinensis*). I have not succeeded in tracing their origin in *Ipomoea*, but these structures are frequently aggregated in the neighbourhood of a nucleus in a manner which suggests their origin from this body (Fig. 20). Two nuclei most often occur in each tapetal cell during the development of the pollen-grains, and these may still be seen as somewhat shrunken, degenerating bodies in stamens which are nearly mature.

From the foregoing account of the development of these pollen-grains it will have been seen that there is no contraction of the protoplast from the pollen-wall at any time, even though the cytoplasm of the pollen-grain is at one stage represented only by a thin, hollow shell of material.

Nevertheless, it is noteworthy that practically the entire growth of the spines and the rodlets takes place after the rudiments of these structures have been separated from direct contact with the protoplasm by the interpolation of the thickening bands of the exine ('mesospore'). That the growth of these structures is considerable will be seen from the fact that the spines increase in length from a rudiment which is too minute for measurement, to a comparatively massive spine with a length of 12 to 14 μ in the mature pollen-grain. It appears to me, therefore, that the growth of these spines and rodlets, which are in contact neither with the pollen-protoplast nor with

the tapetal cytoplasm, is of quite the same character as the growth of the entire membranes of *Isoëtes*, *Selaginella*, *Oenothera*, or *Mirabilis*. The present instance may not at once appear so striking as these latter cases are, but it is no less an interesting and clear example of the growth of a portion of the cell-membrane in entire independence of the direct influence of the living protoplasm.

In the case of the spines and rodlets of *Ipomoea*, as in that of the membranes mentioned above, the origin and first differentiation takes place under the direct control of the protoplasm, but, once formed, the further growth may continue and, moreover, maintain throughout the characteristic shape and structure of the part, quite independent of any immediate guidance from the living protoplast, provided only the material necessary for this growth is forthcoming.

In conclusion, I desire to express my indebtedness to the Government Grant Committee of the Royal Society for the loan of a Zeiss $\frac{1}{12}$-inch apochromatic objective (1·40 aperture), which has been invaluable throughout this research.

Summary.

1. At the conclusion of the second meiotic division the chromosomes remain distinguishable for a short time after the reconstruction of the daughter nuclei, but subsequently their substance becomes completely dispersed over the linin-reticulum.

Chromatic aggregations also occur in many of the nuclei of the anther tissues, notably in those of the young vascular bundle, but the size and number of these aggregations are quite inconstant.

2. The pollen-wall, when it first becomes recognizable, is a single, delicate membrane in which no structure can be distinguished.

3. The exine of slightly older pollen-grains consists of an outer lamella, upon the inner face of which is deposited a network of thickening bands. At the angles of the meshes of this reticulum the rudiments of the future spines already occur. Between the thickening bands and the outer lamella a narrow unstained space or layer can be seen; this marks the position in which the rodlets of the older pollen-grains are developed.

4. The outer surface of the pollen-grain is at first quite smooth. The spine rudiments appear to project towards the pollen-cavity, so that they push the thickening bands inwards at these points into a series of short, internal spinous structures, but they do not extend beyond the outer surface of the grain.

5. During the earlier stages of development, whilst the layers of the special wall and the pollen-wall are being initiated, kinoplasmic fibrils connect the nuclear membrane with the 'Hautschicht' of the pollen-protoplast. Influences of some kind are probably passing along these fibrils

from the nucleus to the 'Hautschicht' which is engaged in the organization of new cell-wall lamellae.

6. In older pollen-grains the spines have grown beyond the surface of the outer lamella of the exine, and the pollen-grain is now distinctly spinous externally. The inner parts or 'roots' of the spines occupy the rodlet layer, and they are double structures each consisting of two prongs. These spines, therefore, differ considerably in their development and structure from the purely superficial ones of such plants as *Althaea* or *Malva*.

7. As the pollen-grains increase in size the protoplast becomes vacuolated and relatively poor in substance, until it is finally reduced to a hollow shell enclosing one enormous central vacuole. In *Ipomoea* there is no contraction of the protoplast away from the pollen-wall, as is observable in the pollen-grains of *Oenothera* or in the spores of *Isoëtes*, &c.

8. The growth of the pollen-protoplast from a hollow shell of cytoplasm to the solid protoplasmic body of the mature pollen-grain is ushered in by changes in the appearance of the nucleus.

This body grows very considerably in size, and there is a relatively enormous increase in the amount of nucleolar matter which it contains.

9. The protoplasm of the older pollen-grain contains a quantity of reserve material. Starch, which in earlier stages was scanty, now occurs in great abundance. Also a material which blackens with osmic acid, and which probably is of a fatty nature, now occurs in some quantity. There is reason to believe that this fatty substance is derived from the tapetal cells, and that it passes from these through the exit pores of the exine into the interior of the pollen-protoplast.

10. The cytoplasm of the small generative cell which is cut off from the large tube cell is almost entirely composed of kinoplasmic fibres.

11. The tube nucleus is large, irregular, and amoeboid in form. It possesses a large nucleolus which is surrounded by a distinct chromatin sheath.

12. An intine develops within the exine. It forms a thin layer over the general surface of the exine, but at each exit pore it attains considerable thickness and protrudes towards the exterior. Its microchemical reactions indicate that it consists of pectic bodies associated with some cellulose.

13. In older pollen-grains the constitution of the exine is much more clearly seen than at earlier stages. It consists peripherally of an outer lamella which is very delicate in structure and perforated by countless little pores or apertures so that its substance is distributed as a delicate reticulum with open meshes. The thickening bands have grown greatly both in thickness and in breadth. They now form together a thick layer (the 'mesospore') perforated by the relatively narrow exit pores for the pollen-tubes. The outer lamella of the exine dips down slightly into the exit pores and covers over the protrusions of the intine at these spots. The

rodlets vary in size, and are usually drumstick-shaped with a part of their knobs just projecting through the perforations of the outer lamella of the exine. The spines are now large (12–14 μ) spindle-shaped structures with the two prongs of their roots lying beneath the outer lamella of the exine and just reaching to the 'mesospore', and their apices passing through the outer lamella to the exterior. Between the rodlets and spine roots a homogeneous, stainable material occurs; this material is not protoplasmic in nature as it does not give the reactions characteristic of this substance (e. g. no xanthoproteic reaction).

14. The tapetal cells do not disintegrate, and must, therefore, be classed with Goebel's 'secretion-tapeta'.

15. Deeply staining fibres and granules occur in the cytoplasm of the tapetal cells during the middle periods of anther development.

16. Almost the entire growth of the rodlets and spines takes place after these have become separated from direct contact with the protoplast by the interpolation of the thickening bands (mesospore). Neither are they in contact with the tapetal or any other cytoplasm. The conclusion may, therefore, be drawn that these structures possess a certain power of growth independent of any direct protoplasmic influence, and, moreover, during this growth they are able to maintain their characteristic form. The growth of the spines and rodlets of *Ipomoea* appears, therefore, to be of quite the same character as that of the entire membranes of *Isoëtes*, *Selaginella*, *Oenothera*, or *Mirabilis*.

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EXPLANATION OF PLATE XIII.

Illustrating Mr. Beer's paper on Spore Development.

All figures refer to *Ipomoea purpurea*, Roth., and were drawn with the aid of the camera lucida. For Figs. 1, 4, 6, 8, 9, 12, 15, 16, and 18, Zeiss' apochrom. objective $\frac{1}{12}$ inch (apert. 1·40) and compens. oc. 8 were employed, whilst for Figs. 5, 7, 11, 13, 14, 17, 19, 20, Leitz's $\frac{1}{10}$ inch object. and compens. oc. 8 were used. Magnification about 1500 and 1100 respectively.

Fig. 1. Telophase of second meiotic division. (*a*), (*b*), (*c*) show gradual vacuolization and dispersal of chromosome material. (*d*) Nucleus from tissue of young vascular bundle showing chromatic aggregates.

Fig. 2. Young pollen-cells surrounded by special walls. × about 640.

Fig. 3. Triradiate middle lamellae of special walls left after disintegration of this wall.

Fig. 4. Young pollen-grain with simple exine. The special wall still encloses the pollen-grain.

Fig. 5. Inner surface view of exine of young pollen-grain.

Fig. 6. (*a*), (*b*), (*c*). Exine of young pollen-grain in section. Same stage as Fig. 5.

Fig. 7. Young pollen-grain soon after its liberation from special walls.

Fig. 8. Older stage of exine than that shown in Fig. 7.

Fig. 9. (*a*) Still older stage of exine. The two prongs or roots of spine are clearly shown. (*b*) Slightly more enlarged view of spine.

Fig. 10. Protoplast of pollen-grain reduced to a hollow shell of substance. × about 480.

Fig. 11. Nucleus of pollen-grain in which the protoplast is just beginning to be reconstructed.

Fig. 12. A cytoplasmic projection into one of the exit pores in the exine. Dark stained material is shown apparently entering the pollen-grain at this point.

Fig. 13. Generative cell of pollen-grain being cut off. Cytoplasm of this cell, consisting chiefly of kinoplasmic fibres, can be seen.

Fig. 14. Generative cell and nucleus at a later stage.

Fig. 15. Tube nucleus of pollen-grain during the reconstruction of pollen-protoplast.

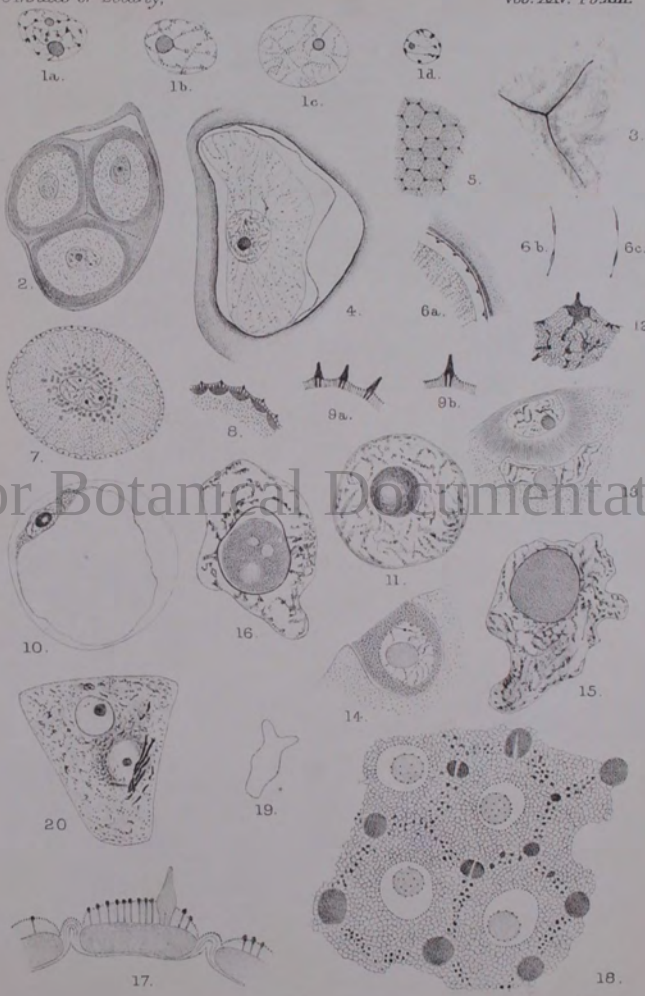
Fig. 16. Similar nucleus to that represented in Fig. 15. Note chromatic sheath round nucleolus has separated from nucleolar substance at one point.

Fig. 17. Wall of a nearly mature pollen-grain.

Fig. 18. The same in surface section.

Fig. 19. Spine from exine with dichotomously branched apex.

Fig. 20. Tapetal cell with deeply staining fibres and granules lying in its cytoplasm.



BEER — SPORE-DEVELOPMENT.

B9a

Studies in Spore Development

Introduction

By Rudolf Beer B.Sc. F.L.S.

During the eighties of last century ~~was a period~~ a great activity was displayed in the investigation of the structure & development of the spores & pollen grains of a large number of plants.

The first impetus to this activity was ~~undoubtedly~~ due to the splendid investigations of Strasburger upon this subject which he published in his treatise "Ueber den Bau & das Wachsthum der Zellhäute" in 1882.[16]

Two years later Dr Hubert Leitgeb issued his studies upon the structure & development of a number of Spores with particular reference to those of the Hepaticae. In 1886 appeared Wille's "Ueber die Entwickelungsgeschichte der Pollenkörner der Angiospermen etc" whilst in 1889 Strasburger published a further series of studies of Spores & pollen grains in Heft II of his Histologische Beiträge.

After this date there was a temporary lull in the ~~desultory~~ investigation of ~~interest displayed~~ if ~~the Subject of~~ Spore ~~morphology~~ histology + until the opening year of the new century the only important contribution to the subject — apart from the interesting micro-chemical work of Mangin — is yet another publication of Strasburger's ~~upon~~ in 1898 upon "Die pflanzlichen Zellhäute"

In 1900 an important ~~book~~ account of the structure & development of the Spores of Isoetes & Selaginella appeared from the pen of Hans Fitting. [5] ~~during the last decade~~ The ~~unexpected feature~~ In this work, ~~full~~ crowded with a wealth of interesting details, probably the most unexpected feature was the demonstration that the Spore-walls could continue their growth although the protoplast of the Spore was not ~~at the~~ in contact with them during this process. Fitting's discovery was very soon confirmed

[2] [4].

in Selaginella by Campbell[3] & a little later by Denke[4]. Miss Lyon[10a] has given a different interpretation of the growth of these membranes but I think her objections have been sufficiently met by Fitting's reply in the Botanische Zeitung [6]. In 1905 I[1] was able to find another case (Oenothera) in which the pollen membranes possess independent powers of growth whilst the protoplast is not in contact with them. In 1908. Tischler[2] observed & added another & most striking example of this phenomenon. In the pollen (grains) of Mirabilis Jalapa the protoplasmic contents degenerate & shrink to a scarcely noticeable quantity & yet notwithstanding this the exine of these grains continues to grow very considerably in thickness & extent. For some time past I have been examining a large number of spores & pollen grains belonging to many species of plants in order to find, if possible, other examples of membranes which are able to continue their growth without the direct cooperation of the protoplasm. I have, upto the present, found no other such striking cases as those of Selaginella, Isoëtes, Oenothera or Mirabilis but a careful examination

of so large a number of different Spores could not fail to bring to light many interesting details which supplement our present knowledge of the subject.
I have already given a description in these pages of two of these Spores (viz Helminthostachys & Riccia) & I now propose to add an account of some other pollen grains & Spores which I have had under observation. I intend to deal with the pollen grains of Ipomoea in the present part.

I Ipomoea

The only account of the finer structure of the pollen-grains of a species of Ipomoea is the very short description given by Strasburger in 1889 [17]. after having furnished a minute account of the spinous pollen of several species of Malvaceae Strasburger devotes a few lines to the pollen-grains of Ipomoea coccinea (Moench.) which appeared to him to be constructed quite after the manner of the Malvaceous type.

I have examined the pollen-grains of Ipomoea purpurea in some detail + as I find that these differ in several respects from the Malvaceous type of pollen I will begin these "Studies" with a description of them.

My material was fixed partly in Flemming's Solutions + also partly in chrom-acetic mixture without osmic acid. So far as possible I have checked my results by comparison with living material examined

in 0.6 % NaCl Solution but the opacity of the Structures did not render this method a very satisfactory one in the present case.
The pollen-mother cells of Ipomoea usually form two or sometimes three longitudinal rows in each pollen sac; they are each surrounded by a wall which gives the reactions of callose & also of pectose & they include a rather large nucleus (about 14 μ in diameter) which contains as a rule, a single large nucleolus & a loose network of fibres. The tapetal cells which form a single layer round the mother-cells, are radially elongated structures which in the majority of cases enclose two nuclei, although cells with three or even four nuclei are met with. The stages of the division of the pollen-mother cells were but poorly fixed in my preparations & with the exception of the telophase of the second meiotic division, which was well shown in my sections, I will make no reference to the subject.

At the conclusion of the second division of the pollen-mother cells the chromosomes retain their individuality for some time after a nuclear wall has been reconstructed + a new nucleolus (or nucleoli) formed in each daughter nucleus (Fig 1a). The chromosomes are distributed throughout the nuclear cavity, but are connected with one another by a delicate linin threadwork. A little later the sharply defined, homogeneous chromosomes become more irregular in outline & apparently vacuolar in structure (Fig 1b) & it is easy to trace the gradual opening out of their substance & its distribution over the linin network until no trace of any individual chromosome can any longer be detected (Fig 1c) (After the division of these nuclei, is finally completed) Nothing can be seen therefore, nothing in the nature of the "pro chromosomes" which Rosenberg, [14,15] Overton [12,13] Laibach [10] + others have described in the "resting" nuclei of various plants.

I have examined the nuclei of the other tissues of the anther for prochromosomes ~~but although chromatic bodies are~~ & in some, notably those of the young vascular tissue, chromatic aggregates are to be seen lying beneath the nuclear wall which resemble in appearance the prochromosomes of other writers (Fig 1d). Their number, however, appears to me to be too inconstant in these cells to ~~have any~~ have the significance of which attaches to true prochromosomes.

There is evidently ~~considerable~~ great variation in the behaviour of the chromosomes of different cells at the conclusion of nuclear division. In some, such as the cases described by Rosenberg & others, the chromosomes appear to retain a large proportion of their material definitely aggregated as clearly distinguishable prochromosomes throughout a prolonged period of rest. In

other cases, such as the pollen-mother-cells of Ipomoea, the chromosomes retain their individuality as distinct bodies for a short, but yet quite definite period + then their substance becomes evenly distributed over the linin reticulum. ~~Whilst in many~~ Finally in many other cells the chromosomes become vacuolated + their substance dispersed over the linin threadwork immediately at the conclusion of mitosis. In such cells such as those of the young vascular tissue of Ipomoea in which an inconstant number of chromatic aggregates occurs it is probable that some of the chromosomes (or part of their substance) may remain visible as distinct bodies for a greater or less time, others may become distributed over the linin at once + lose their visible individuality whilst others may become vacuolated + broken up into two or more smaller but still recognisable bodies. ~~The chromatic aggregations may also be~~ partly or entirely ~~of a secondary character~~ associated with the substance of the nucleolus + have no direct connection with the chromosomes

The tetrads of young pollen-grains become surrounded by massive mucilaginous walls. Upon the periphery of each tetrad-group is a granular deposit which stains with bismarck brown but not with aniline-blue or corallin-soda. This is probably the remains of the primary wall which separates the sporogenous cells from one another. Within this a distinct, often rather massive layer is seen which possesses the staining properties characteristic of Mangin's callose + pectose. This is the mother-cell wall already referred to above. Within this again is another mucilaginous wall which also gives the reactions of callose + pectose and which envelopes the young pollen grains + separates them from one another. From Strasburger [19] has For this innermost wall Strasburger has recently suggested the convenient name of Special-wall to replace the old term Special-mother-cell wall with its false implication (Fig 2)

In microtome sections which have been stained either with aniline blue or congo red three radiating lines (really lamellae) of a granular appearance can be seen to traverse the middle of the special wall (Fig 2). These granular bands are the first lamellae which are formed at the conclusion of the division of the mother-cell. In sections stained with Heidenhain's Iron-alum haematoxylin & bismarck brown these lamellae are often quite unstained & appear as colourless clefts or lines in the middle of the brown special-wall substance. Mangin [1] has described similar tri-radiate lines of granules in the case of Althaea rosea & he states that they are nitrogenous in character. I was unable to determine their chemical nature in the case of I pomoea.

At the time when the callose walls break down & set the pollen grains at liberty it is often seen that the tri-radiate lamellae contain

(1) Mangin Bull. Soc. Bot. de France T. 56 1889 p. 224

to exist for some time in the midst of the flocculent material derived from the rest of the wall. (753)

The callose pulvar layers of the special-wall which immediately envelope the pollen grains ~~are~~ & which are the latest parts of this wall to be formed are denser than those in the neighbourhood of the granular lines.

There is evidence, ~~therefore~~, that the special-wall of Ipomoea possesses a laminated constitution.

The young pollen grains of Iponoea surround themselves with a wall of their own - the Exine. This is deposited by the pollen protoplast as an extremely delicate layer upon the inner face of the callose - pectose wall which surrounds it. From the first it is marked off as an independent structure ~~into~~ from the callose - pectose special wall & there can be no doubt that it is a new membrane & not one derived from the transformation of the innermost lamellae of the special wall.

In its Earliest stages it is an ~~extremely~~ Exceedingly delicate membrane which is too thin to permit any Structure to be seen in it even with the highest powers of the microscope & in the most delicate microtome sections. ~~(Fig 4 Fig~~

~~When a structural differentiation can first be~~ later in Fig 4 . a young pollen grain is represented lying within the special wall. Here the pollen - protoplast has contracted

under the influence of the reagents & the young exine has also separated from the special-wall from the same cause. Under these circumstances the newly developed exine can be seen exceptionally well as an independent membrane of great tenacity. In somewhat older pollen grains a structural differentiation of the exine can be detected - which even at this early stage exhibits some complexity. The exine can now be seen to consist of an outer lamellum upon the inner face of which is deposited a network of thickening bands. At the angles of the meshes of this network the rudiments of the future spines already occur. Between the thickening bands & the outer lamellum a narrow cleft or unstained space can be seen & this is the position in which the rodlets of older pollen grains are developed.

Fig 5 shews the inner ~~surface~~ face of the Exine at this stage in Surface View. The more deeply staining System of thickening bands are seen to form a ~~network~~ reticulum with polygonal (mostly hexagonal) meshes upon the lighter outer layer of the exine. At the angles of the network the Spine-rudiments are seen as deeply coloured dots. Fig 6 represents the same stage in Section. Here the still very delicate thickening bands are seen lying upon the inner surface of the outer lamellum but separated from it by an unstained cleft or interval. ~~Below the~~

Fig 6 c represents the same stage in Section. Here the alternation of thicker areas, where the thickening bands lie, with thinner intervals is seen. In very delicate microtome sections the Separation of the thickening band from the outer lamellum of the Exine by a clear, unstained ~~cleft or interval~~ space or layer can readily be made out (Fig 6 b).

Where the Section has passed through the Spine-rudiments the appearance is somewhat different. In Fig 6a it will be seen that the thickening band (by the colourless layer) appears to be pushed inwards at each Spine-rudiment so as to form an internal Spine. The Spine rudiment itself appears as a deeply stained (fuchsin) particle at the apex of each of these projections. The external surface of the Exine is still completely flat & smooth.

The mode of development which these component parts of the exine follow is a difficult if not impossible, ~~task~~ matter to decide [illegible] with certainty. A careful study of my preparations inclines me to believe that the outermost lamellum of the ~~thicken~~ system of thickening bands are successive developments secreted one after the other by the pollen-protoplast. The thin, apparently structureless membrane of such stages as that represented in Fig I believe

The mode of development which these component parts of the exine follow is a difficult matter to decide with certainty. There can be little doubt, I think, that the outermost lamellum & the system of thickening bands are successive developments secreted by the pollen-protoplast one after the other. The thin structureless membrane of such stages as that represented in Fig 4 I believe to correspond to the outermost lamellum alone. Upon this the bands of thickening are laid down by the protoplast in somewhat older pollen grains. At first these bands are so thin & faintly marked as to appear as little more than shadowy tracings upon the inner face of the membrane but they rapidly gain in distinctness as development proceeds & new material is added to them by the protoplast. Exactly how & when the spine-rudiments & the rodlets are first

developed is a more difficult problem to determine. The impression which I have gained from the study of my preparations is that the clear space which is seen in the sections to lie between the thickening bands & the outer lamellum represents a third & distinct layer of substance (with little affinity for stains) deposited by the protoplast previous to the development of the thickening bands. This layer subsequently becomes differentiated into the spines & nodules. This interpretation of the layers of the exine of Ipomoea would be more or less in accordance with the views expressed by Strasburger [18] in the cases of Knautia & Althaea & by Tischler [21] for Mirabilis Jalapa.

It is of course possible that the layer containing the nodules & spines may not become differentiated later, after the thickening bands have already been deposited, but I think this is unlikely both from the appearances in the present case & from analogy with what occurs in other plants.

the views expressed by Strasburger for the cases of *Knautia* & *Althaea* & by Tischler for *Mirabilis Jalapa*. It is, also conceivable, however, that after the outer lamellum & the thickening bands have been ~~developed~~ successively deposited a separation may occur between the two & that the rodlets & spines might grow into this space either from the outer lamellum above or from the thickening band below but I consider this very unlikely to judge from the appearance of the spines & rodlets in the older pollen grains ~~[illegible]~~

The thickening bands quickly increase in both thickness & ~~[illegible]~~ breadth as fresh material is added to them by the protoplast. During the early stages the substance of the thickening bands appears to be soft & mucilaginous; so that their outer margin is ill defined & encroaches upon the clear spaces or layers referred to above so that these becomes difficult to distinguish & the spine-rudiments have the appearance of being embedded in the substance of the bands. (Fig 7)

The external surface of the pollen grain still remains smooth as the spine rudiments do not yet project above its surface.

It may be noted that during the time that the various layers of the special wall + of the pollen wall are being laid down by the protoplast kinoplasmic fibres can clearly be distinguished running between the nuclear membrane + the "hautschicht" at the periphery of the protoplast (Figs 4, 6 & 7). These fibrils can be traced back to the kinoplasmic radiations which surround the nuclei during the telophase of the second meiotic division. The persistence of fibrillar differentiations of this kind is by no means uncommon during the earlier stages of the development of the pollen grain + I have met with it in several other plants besides Ipomoea.

It seems quite probable that influences of some kind are distributed along these fibrils from the nucleus to the "hautschicht" which is taking an

active part in the formation ~~of the new cell-wall~~ of the new cell-wall lamellae.

Up to the present the young pollen grains have remained enclosed within the Special-wall. Now, however, these walls break down into a diffuse, flocculent material which fills the cavity of the anther-loculus. The tri-radiate middle-lamellae of the Special-walls, which we previously recognised as granular lines in the middle of the callose-pectose walls, often remain intact for some time longer & can be seen lying in the midst of the flocculent material derived from the degeneration of the rest of these walls. (Fig 3).

Soon after this time the Spine-rudiments ~~begin~~ have grown sufficiently ~~in~~ centrifugally ~~directions~~ to project very slightly above the outer surface of the pollen grain & to give this a waxy appearance. Before long they project ~~so far~~ far enough beyond the ~~periphery~~ periphery of the Exine

to give this a distinctly spinous appearance. At about this period the "rodlets" can first be clearly observed as minute deeply stained structures lying in the position of the clear ~~line~~ space or layer noticed at an earlier stage between the outer lamellae of the Exine & the thickening bands. Both the "rodlets" & the spines now stain ~~at this period~~ much more deeply than the rest of the exine & they are, therefore, very clearly distinguished in the Sections (Fig 8)

At ~~about~~ this time the Kinoplasmic fibres running between the nuclear membrane & the "Hautschicht" become obscure either because they are hidden by the accumulation of metaplasmic materials in the protoplast or because they merge into & become lost in the alveolar substance of the cytoplasm.

In rather older pollen grains the relation of the parts of the Exine to one another becomes much clearer. The thickening bands have increased

greatly in thickness + have become much broader so that the thin areas of the exine between these bands are now reduced to a series of pores or narrow channels which represent the exit-pores for the future pollen tubes. ~~The rodlets have become much longer~~ Moreover the substance of the bands appears to have undergone a change for these are no longer diffuse + ill defined at their inner margins but they are now sharply marked off from the distinct "rodlet layer". ~~which~~ The spines have grown greatly in size. They are still limited to the angles of the network of thickening bands + they are now seen to be spindle shaped with their points projecting for some distance beyond the still very delicate outer lamellum of the exine + their "roots" occupying the whole thickness of the rodlet layer. These "roots" moreover are seen to be double Each

consisting of two prongs (Figs 9a+b). A surface view of the pollen grains at this stage shows that the rodlets are limited to the positions overlying the hexagonally arranged thickening bands & that they themselves, therefore, form an hexagonal figure when viewed from above. This is shown clearly in Fig 18 (although this represents an older pollen grain in surface view.

The protoplast of the pollen grain which completely fills the pollen cavity, has meanwhile become much poorer in substance & more vacuolated than at earlier stages. As the pollen grain has increased from about 32μ to about 45μ in diameter the decrease in protoplasmic density is, at any rate partly, due to its substance being distributed over a larger area but I believe that there is also a real loss of substance by the protoplasm which has contributed some material to the

growing membranes. The nucleus has only increased very slightly in size; the average of a number of measurements shewed only an enlargement of about 1.2 μ (from 10 μ of an earlier stage to 12 μ now). The nuclear reticulum has become somewhat coarser + stains more deeply; one, two or often more rather small nucleoli may occur.

The pollen grain continues to increase in size + its wall grows both in surface + in thickness, in proportion as the growth ~~continues~~ proceeds the protoplast continues to become more vacuolated + poorer in substance although it never contracts away from the pollen-wall as in Oenothera. In pollen grains which measure 70 μ in diameter the cytoplasm encloses a number of large vacuoles + the nucleus, which now measures about 14 μ in diameter contains one or more nucleoli + a rather scanty ~~granular~~ reticulum. By the time the pollen

grains have reached 80 or 90 μ in diameter the cytoplasmic lamellae which separate the large vacuoles from one another have become broken down + the protoplast is reduced to a hollow shell with a single large vacuole occupying its entire centre. This cytoplasmic shell consists of little besides a "hautschicht" except in the immediate vicinity of the nucleus where some granular cytoplasm still remains Fig 10. The nucleus is now a flattened body measuring about 20 μ by 10 μ across its greatest & least diameters. It encloses a rather scanty somewhat faintly stained arrangement of threads & one, two or more nucleoli of large size. The great increase of nucleolar matter is certainly the most striking change in the nucleus from its earlier stages; these larger nucleoli may measure as much as 8 μ across. Not infrequently the interior of the

nucleoli has a vacuolar appearance. The alteration in the appearance of the nucleus which is just beginning to become evident ushers in the process of protoplasmic reconstruction. The cytoplasmic shell is seen to become slightly thicker + the granular cytoplasm which ~~was seen~~ had been reduced to one small area near the nucleus can now again be observed as a thin layer all round the inner surface of the hollow protoplast. In the meanwhile the protoplast nucleus has increased in size to as much as 30 μ by 20 μ in its longest + shortest diameters. The pollen grain itself still measures 90 μ across. This nucleus encloses one (rarely two) extremely large nucleolus which on an average measures about 12 μ in diameter. The fibrils which traverse the nuclear cavity have become much more numerous; they are

finely granular in appearance & diffuse, ill defined & irregular in outline. (Fig 11) The protoplasmic shell continues to grow in thickness & before long the single central ~~Vacuole~~ vacuole becomes bridged across by one or two cytoplasmic lamellae which divide it up into a few large vacuoles. These become progressively smaller as the protoplasmic lamellae grow more massive & more numerous. Starch, which hitherto was present in only in comparatively small quantities now occurs in great abundance. Granules, or more probably droplets, & irregular masses of material which are ~~stained~~ black in my preparations stained with Heidenhain's haematoxylin also accumulate in the cytoplasm of the pollen grain. The distribution of this dark-staining material in the pollen-protoplast is of some interest. It is usually rather densely collected in the little, peripheral

finger-like {cytoplasmic processes} which project into the exit-pores of the pollen-~~tube~~-wall. From these points this material can be seen to spread ~~out~~ out irregularly ~~in~~ into the interior of the protoplast (Fig 12). It has the appearance of a material, ~~which~~ derived from without, which is making its entrance through the exit-pores of the pollen wall, which is then taken up by the little pseudopodium-like processes of the protoplast + which from these points becomes diffused throughout the cytoplasm of the pollen grain. I have been able to obtain little information with regard to the chemical nature of this substance. From the fact that it is blackened by Osmic acid in Flemming's solution it is ~~quite~~ probable that the material is of a fatty nature but beyond this I can say nothing at present.

It may be noted that just about the time when this dark-colored material is making its

appearance in the pollen-protoplasts a number of vacuoles of varying sizes are formed in the tapetal cells + that these vacuoles are filled with a material which is also darkened by osmic acid. I have not succeeded in tracing the darkened material out of the tapetal cells into the cavity of the anther + to establish a direct connection with the similarly blackened substance in the pollen-protoplast but ~~it will not be advisable that such a connection is at least highly probable~~.

Such a relationship between the two appears quite likely. Moreover, the tapetal cells can be seen to undergo a loss in the total amount of substance they contain. These facts taken together suggest that the pollen-protoplasts are growing + storing reserve bodies in their substance at the expense of materials derived, at any rate in part, from the tapetal cells. During the earlier stages of the growth of the

pollen-protoplast we find that its nucleus divides & that the very unequal cell division which follows cuts off a small generative-cell from the large tube-cell. The cytoplasm of the generative cell is almost entirely composed of ~~radiating~~ Kinoplasmic fibres radiating from its nucleus. (Fig 13) In older generative-cells the fibrillar constitution of the cytoplasm gives place to a dense, hyaline structure.

A distinct plasma membrane limits the generative-cell peripherally, but no cell-wall is developed (Fig 14)

The nucleus of the generative-cell measures about 14μ in diameter & contains a comparatively large nucleolus & a rather loosely arranged system of fibres.

The tube-nucleus is large, irregular or even amoeboid in outline & is distinguished by the enormous nucleolus & the system of deeply staining chromatic threads which it contains (Fig 15) & Amoeboid tube-nuclei have been described

in several other plants; for instance in Elodea canadensis by Wylie [22].
The nucleolus of the tube-nucleus is surrounded by a sheath of chromatin & there is here no signs of the clear space (Heller Hof) between the nucleolus & the chromatic reticulum of the nucleus which several writers have described. (Fig 15) In such cases as that represented in Fig 16 where a slight contraction of the nucleolar substance has taken place at one spot the relation between the nucleolar material & the chromatic sheath is particularly well seen. Martin Heidenhain as long ago as 1892 (& again in 1904) [9] described chromatic shells of this kind enveloping the nucleolus in several animal tissues & his observation has been confirmed by other zoologists.

Cavara's observations upon nucleoli may also be recalled in this connection. This author described the nucleoli of higher plants as consisting of two parts: an external chromatic layer & an inner mass of plastin. ~~C. T. Hotter obtained somewhat similar results by employing special culture methods for the plants.~~

During this period of protoplasmic growth an intine has been formed over the inner surface of the pollen-wall. This layer is very thin over the general surface of the pollen grain but at each exit-pore it is greatly thickened & protrudes towards the exterior. When it is thickened the intine can clearly be seen to be composed of a number of lamellae which suggests that its growth has taken place by apposition of successive layers of

material. An extension of the delicate outer ~~lamellum~~ layer of the exine covers the external surface of each protrusion of the intine at the exit pores of the pollen grain (Fig 17). The intine stains, although not very intensely, with those dyes which are characteristic of pectic bodies. Treated with calcium chloride - Iodine solution it gives at first no reaction but after remaining in the solution for some days it is found to have coloured faintly violet. A preliminary boiling with dilute acid & alkali, according to Mangin's method, yielded no clearer cellulose reaction with the Iodine reagents. From these reactions it may be concluded that the intine consists of pectic bodies associated with a little ~~with~~ cellulose. The exine has meanwhile grown ~~considerable~~ in thickness & the relation of its parts to one another can now be ~~more~~ very clearly seen. ~~The thickening bands which now~~ ~~together~~ form ~~a massive, elast~~

The ~~the~~ System of thickening bands now forms a massive layer only perforated by the exit-pores; it ~~this~~ gives the reactions of a cuticularized membrane.

The thickening bands of the exine have increased greatly in breadth & thickness so that they now form a massive layer only perforated by the relatively small exit-pores. ~~If it be desirable to~~ This layer might be called the "mesospore" according to Zetting's terminology, possesses the reactions of a cuticularized structure. (Figs 17 ~~18~~)

[7]

The outermost lamellum of the exine (which we already saw as an extremely delicate membrane at an early stage) ~~the ectexine~~ still remains very thin & ~~but~~ it can now be seen to possess an open structure perforated by countless little apertures which give it the appearance of a very fine reticulum in surface views. This perforated structure of the lamellum is well

seen in Fig 18. & in Section in Fig 17 & 19.
At the exit-pores this reticulate layer dips down & covers over the protrusion of the intine.
As at an earlier stage we still find that the rodlets are limited to an hexagonal system of bands corresponding to the originally hexagonal disposition of the thickening bands (Fig 18). at the angles of each hexagon is usually a spine. Both the spines & the "heads" of the rodlets pass through the perforated outer lamellum to reach the outer surface.

The spines are spindle shaped structures, their internal portions or roots are composed of two prongs as already seen at an earlier stage. Fig 18 shews that these prongs have an hemispherical outline in transverse section. A stainable, homogeneous material lies beneath the reticulate outer lamellum

between the rodlets under the reticulate outer lamellum.

In Sections which have been mounted in a drop of glycerine containing a little methylene blue & fuchsin mixture an interesting differentiation of the parts of the pollen wall can be seen. The intine colours light red, the "mesospore" is blue, the reticulate outer lamellum of the Exine, the Spines & the rodlets are green whilst the homogeneous into-reticulate substance (between the rodlets) colours deeply red.

The mature Spines of the Exine measure between 12 & 14 μ in length, they are usually fusiform in outline although I have occasionally found them with a dichotomously branched apex (Fig 19).

The rodlets vary a good deal in size, their shape is usually like that of a drumstick with a part of the knob or head just projecting

~~just~~ through the perforated outer lamellum of the testa (Fig 17)

The tapetal cells do not break down + scatter their contents between the pollen grains but they remain surrounded by a membrane until the last. This tapetum, therefore, belongs to the "Secretion - tapeta" of Goebel.

Deeply staining fibres & granules occur in the cytoplasm of the tapetal cells of Ipomoea during the middle period of anther development. These are most probably similar to the chromidial structures which have been described in the tapeta of several other plants (Nymphaea alba, Oenothera, Ribes, Lilium Martagon, Iris germanica, Syringa chinensis). I have not succeeded in tracing their origin in Ipomoea but they are frequently aggregated in the neighbourhood of a nucleus in a manner which suggests their origin from this body (Fig 20). Two nuclei most often occur in each

tapetal cell during the development of the pollen grains & these may still be seen as somewhat shrunken, degenerating bodies in stamens which are nearly mature.

From the foregoing account of the development (of these pollen grains) it will have been seen that there is no contraction of the protoplast from the pollen-wall at any time even though the cytoplasm of the pollen grain is at one stage represented only by a thin, hollow shell of material.

~~In these pollen grains cannot~~

Nevertheless it is noteworthy that ~~almost~~ the practically the entire growth of the spines & the rodlets takes place after the rudiments of these structures have been separated from ~~the~~ direct contact with ~~of~~ the protoplasm by the interpolation of the thickening bands of the exine (mesospore). ~~That the~~ (that the) growth of these structures is

~~considerable will be seen as the Spines increase in length from a rudiment which is too small for direct measurment~~

considerable will be seen from the fact that the Spines increase in length from a rudiment which is too minute for measurment to a comparatively massive Spine with a length of 12 to 14 μ in the mature pollen grain. It appears to me therefore probable that the growth of these Spines + rodlets, which are in contact neither with the pollen protoplast nor with the capital cytoplasm, is of quite the same character as the growth of the entire membranes of Isoetes, Selaginella, Oenothera or Mirabilis. The present instance may not at once appear so striking (as many latter cases are) but it is no less an interesting + clear example of the growth of a portion of the cell-membrane in entire independence of the direct influence of the lining protoplasm. In the case of the Spines + rodlets of Ipomoea, as in

that of the membranes mentioned above, the origin + first differentiation takes place under the direct control of the protoplasm but, once formed the further growth may continue & moreover maintain throughout the characteristic shape + structure of the part. quite independent of any immediate guidance from the living protoplast provided only the material necessary for this growth is forthcoming.

In conclusion I desire to express my indebtedness to the Government Grant Committee of the Royal Society for the loan of a Zeiss 1/12" oil apochromatic objective (1.40 aperture) which has been invaluable throughout this research.

Summary

(1) At the conclusion of the second meiotic division the chromosomes remain ~~visible~~ distinguishable for a short time after the reconstruction of the daughter-nuclei but subsequently their substance becomes completely dispersed over the linin-reticulum. Chromatic aggregations also occur in the many of the nuclei of the anther-tissues, notably in those of the young vascular-bundle, but the size & number of these aggregations is quite inconstant.

(2) The pollen wall, when it first becomes recognisable, is a single, delicate membrane in which no structure can be distinguished.

(3) The exine of slightly older pollen grains consists of an outer lamellum upon the inner face of which is deposited a network of thickening bands. At the angles of the meshes of this reticulum the rudiments of the future spines already

occurs. Between the thickening bands & the outer lamellum a narrow unstained space or layer can be seen; this marks the position in which the rodlets of the older pollen grains are developed

(4) The outer surface of the pollen grain is at first quite smooth. The spine-rudiments appear to project towards the pollen-cavity so that they push the thickening bands inwards at these points into a series of short internal spinous structures but they do not extend beyond the outer surface of the grain.

(5) During the earlier stages of development, whilst the layers of the Special-wall & the pollen-wall are being initiated, Kinoplasmic fibrils connect the nuclear membrane with the "Hautschicht" of the pollen-protoplast. Influences of some kind are probably passing along these fibrils from the nucleus to the "Hautschicht" which is engaged in the organisation of new cell-wall layers.

(6) In older pollen grains the spines have grown beyond the surface of the outer lamellum of the exine + the pollen grain is now distinctly spinous externally ~~upon its external surface~~. The inner parts or "roots" of the spines occupy the nodlet layer + they are double structures each consisting of two prongs. These spines, therefore, differ considerably in their development + structure from the purely superficial ones of such plants as Althaea or Malva.

(7) As the pollen grains increase in size the protoplast becomes vacuolated + relatively poor in substance until it is finally reduced to a hollow shell enclosing one enormous central vacuole. In Ipomoea there is no contraction of the protoplast away from the pollen-wall as is observable in the pollen grains of Oenothera or in the spores of Isoëtes &c.

protoplast away from the pollen-wall as is observable in the pollen grains of Aeschynanthus or the spores of Isoetes etc.

(8) The growth of the pollen-protoplast from a hollow shell of cytoplasm to the solid protoplasmic body of the mature pollen grain is ushered in by changes in the appearance of the nucleus. ~~There is an enormous increase in the nucleolar matter of the body contained in the nucleus~~ This body grows very considerably in size & there is a relatively enormous increase in the amount of nucleolar matter which it contains.

(9) The protoplast of the older pollen grain contains a quantity of reserve material. Starch, which in earlier stages was scanty, now occurs in great abundance. Also a material which blackens with osmic acid & which is probably of a fatty nature now

occurs in some quantity. There is reason to believe that this fatty substance is derived from the tapetal cells + that it passes from these through the exit-pores of the exine into the interior of the pollen-protoplast.

(9)(10) The cytoplasm of the small generative cell which is cut off from the large tube-cell is almost entirely composed of kinoplasmic fibres.

(11) The tube-nucleus is large, irregular + amoeboid in form. It possesses a large nucleolus which is surrounded by a distinct chromatin-sheath.

(12) An intine develops within the exine. It forms a thin layer over the general surface of the exine but at each exit-pore it attains considerable thickness + protrudes towards the exterior. Its micro-chemical reactions indicate that it consists of pectic bodies associated with some cellulose.

(13) In the older pollen grains the constitution of the Exine is much more clearly seen than at earlier stages. It consists of an outer lamellum which is very delicate & structureless perforated by countless little pores or apertures. The substance of this delicate lamellum is distributed as a reticulum with open meshes. The thickening bands have grown greatly both in thickness & in breadth. They now form together a thick layer (the mesospore) perforated by the relatively narrow exit-pores for the pollen tubes. The outer lamellum of the Exine dips down slightly into the exit-pores & covers over the protrusions of the intine at these spots. The rodlets vary in size & are usually drumstick-shaped with a part of their knobs just projecting through the perforations of the outer lamellum of the Exine. The spines are now

large (12–14μ), Spindle shaped structures with their two prongs of their roots lying beneath the outer lamellæ of the exine & just reaching to the "mesospore" & their apices passing through the outer lamellæ to the exterior. Between the rodlets & spine-roots a homogeneous, stainable material occurs; this material is not ~~does not give the reactions of Cytoplasm (e.g. no Xanthoprotein reaction) appear to be~~ protoplasmic in nature as it does not give the reactions characteristic of this substance (e.g. no Xanthoprotein reaction).

(14) The tapetal-cells do not disintegrate & must, therefore, be classed with Goebel's "Secretion-tapeta".

(15) Deeply staining fibres & granules occur in the cytoplasm of the tapetal cells during the middle periods of anther development.

(16) Almost the entire growth of the rodlets & spines takes place after these have become separated from the direct contact of the protoplast by the interpolation of the thickening bands (mesospore). Neither are they in contact with the tapetal or any other ~~protoplasm~~ cytoplasm. The conclusion ~~must~~ may, therefore, be drawn that these structures possess a certain power of growth independent of any direct ~~cytoplasmic~~ protoplasmic influence & ~~that~~ during this growth they are able to maintain their characteristic form. The growth of the spines & rodlets of Ipomaea ~~seems~~ appears, therefore, to be of quite the same character as that of the entire membranes of Isoetes, Selaginella, Oenothera or Mirabilis.

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Explanation of Plate

All figures refer to *Ipomoea purpurea* + were drawn with the aid of the camera lucida. For Figs 1, 4, 6, 8, 9, 12, 15, 16 + 18 — Zeiss' Apochrom. objective 1/12 imk (apert. 1.40) + Compens. oc. 8 were employed whilst for Figs 5, 7, ~~8~~, 11, 13, 14, 17, 19, 20 Leitz's 1/10" imk objekt + Compens. oc. 8 were used.

~~Figs 2~~

Fig 1. Telophases of second meiotic division.
(a), (b), (c) Shew gradual vacuolisation + dispersal of chromosome material.
(d) Nucleus from tissue of young vascular bundle shewing chromatic aggregates.

Fig 2. Young pollen-cells surrounded by special walls × about 640.

Fig 3. Tri-radiate middle lamellae of special walls left after disintegration of this wall.

Fig 4. Young pollen grain with simple exine. The special wall still encloses the pollen grain.

Fig 5. Inner surface view of Exine of young pollen grain.

Fig 6. (a), (b), (c). Exine of young pollen grain in section. Same stage as Fig 5.

Fig 7. Young pollen grain soon after its liberation from special walls.

Fig 8. Older stage of exine than that shewn in Fig 7

Fig 9. (a) Still older stage of exine. The two prongs or roots of spine are clearly shewn

(b) slightly more enlarged view of spine.

Fig 10. Protoplast of pollen grain reduced to a ~~hollow~~ hollow shell of substance K. X about 480

Fig 11. Nucleus of pollen grain in which the protoplast is just beginning to be reconstructed.

Fig 12. A cytoplasmic projection into one of the exit-pores in the exine. Dark stained material is shewn apparently entering the pollen grain at this point.

Fig 13. Generative cell of pollen grain being cut off. Cytoplasm of this cell consisting chiefly of kinoplasmic fibres can be seen.

Fig 14. Generative cell & nucleus at a later stage.

Fig 15. Tube-nucleus of pollen grain during the reconstruction of pollen-protoplast.

Fig 16. Similar nucleus to that represented in Fig 15. Note chromatic sheath round nucleolus has ~~contracted away~~ separated from nucleolar substance at one point.

Fig 17. Wall of a nearly mature pollen grain.

Fig 18. The same in surface section.

Fig 19. Spine from ~~exo~~ exine with dichotomously branched apex.

Fig 20. Tapetal-cell with deeply staining fibres & granules lying in its cytoplasm.