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On the development of the pollen grain and anther of some Onagraceae.

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With 3 Plates.

The striking character of the pollen grains of the *Onagraceae* has attracted the attention of botanists from a very early date.

Already in 1830 Purkinje examined and figured the pollen of several species („De cellula, Antherarum Fibrosis etc." Vratislaviae 1830) and since that time Hugo von Mohl, Fritsche, Schacht, Nägeli, Luerssen, Tschistiakoff, Sachs, Wille and Strasburger, as well as others have all paid greater or less attention to this subject. By far the most detailed account which we posses is that of Strasburger embodied in his two memoirs upon the cell-wall (1. „Über den Bau und das Wachstum der Zellhäute" 1882, pp. 95—100. 2. „Über das Wachstum vegetabilischer Zellhäute" 1889, pp. 36—46).

In spite of this attention our knowledge of the development of these anthers is still incomplete and it was the purpose of the present research to re-examine the subject and, if possible, to add a few details to the existing accounts.

The species which have been examined are *Oenothera longiflora*, *O. biennis* and *Gaura Lindheimeri*. *Epilobium tetragonum* and *E. montanum* have also been examined but less thoroughly.

The early development of the anther takes place in quite the usual manner. A single longitudinal row of hypodermal cells (the archesporium) divide into an inner series of primary sporogenous cells and an outer row of primary parietal cells [1].

In the latter a succession of periclinal divisions follow one another until, usually, four layers of cells separate the column

[1]) The terminology used here in that given in Coulter and Chamberlains „Morphology of Angiosperms" 1903, p. 33.

of primary sporogenous cells from the epidermis. The outermost parietal layer is the endothecium or fibrous layer; within this follow (usually) two „middle layers" and finally, adjoining the sporogenous tissue, the tapetum becomes differentiated.

As is well known the primary sporogenous cells of *Gaura* form a single longitudinal row. Subsequently certain of these sporogenous cells become „sterile" and, by their division, form transverse septa, here and there, across the anther. The formation of these septa in certain members of the *Onagraceae* has already been described by Barcianu (Inaug.-Diss. Leipzig 1874 „Unters. über d. Blütenentwick. d. *Onagraceae*" p. 21) and by Bower (Studies in the Morphology of spore-producing members. II. *Ophioglossaceae* 1896, p. 1).

Large raphide-sacs occur in the connective of all the species examined. The bundle of crystals of each sac is enveloped in a mucilage which stains violet with a mixture of methylene blue and fuchsin and pink with rutherium red. These reactions indicate a pectic body. The crystals and their mucilage sheath do not fill the entire sac but the space which is left between them and the wall of the sac is occupied by a material which has often a reticulate structure. In Heidenhain's Iron-haematoxylin the mucilage sheath becomes black whilst the reticular investment remains uncoloured (Fig. 14). No starch and no plastids were ever seen in the sacs which enclose raphides. Warming in his description of the anther of *Epilobium angustifolium* (Unters. über pollenbildende Phyllome und Kaulome Bonn 1873, p. 23) calls attention to certain large, ellipsoidal cells which he in the connective but the nature of which he left undetermined. These cells are the raphide-containing sacs mentioned above which reach quite a remarkable development in the species of *Epilobium*.

In *Oenothera biennis* and *O. longiflora* tannin also occurs in the anther, both in the epidermis and in a varying number of cells of the connective. On each side of the anther, along the line of future dehiscence, a longitudinal band of epidermal cells always remains free from tannin. The cells of these two lateral, tannin-free bands soon ceases to grow and become stretched and flattened by the enlargement of the anther. Beneath each of these two lines of peculiar epidermal cells a longitudinal air-passage is formed at a very early stage [1]). This passage arises, in the first place, by a separation of cells from one another at these spots but subsequently the cavity is enlarged by the cells bordering upon the space becoming flattened and destroyed by the growth of the anther.

In some anthers a curious development of the cell-walls bordering upon the air-passage was observed. The cell-walls in question become greatly thickened and cuticularised in a manner

[1]) Some time before the appearance of the callose mother-cell walls.

which was quite similar to that found in the walls of the pollen grains themselves. The air-passage in these anthers was therefore completely shut off by a continuous mantle of thick, cutinised membranes. The thickening and cuticularisation of these walls had taken place very early in the history of the anther long before the pollen-walls themselves had undergone such changes and indeed before the pollen-wall had put in its appearance at all (Fig. 1).

Stomata occur upon the anther but they are not very abundant. In anthers at about the time when the pollen-mother-cells are established the development of the stomata can be readily followed. It is seen that an initial cell is cut off from certain of the meristematic superficial cells of the anther and this becomes the direct mother-cell of the stoma (Figs. 2, 3 and 4). Starch can nearly always be found in the guard cells of the stoma although the other epidermal cells are quite free from this substance[1]).

I have examined the anthers of *Gaura Lindheimeri* soon after the primary sporogenous cells have become definitely established by means of the cell-wall reagents recommended by Mangin.

I find that both pectic bodies and cellulose are present in the walls of the anther-cells at this time but that the cellulose is ordinarily masked by the pectic constituent. It is only after treating the sections with dilute acid followed by the action of dilute alkali that the cellulose can be clearly demonstrated. The walls of the sporogenous cells and of the tapetum contain less cellulose than the other regions of the anther.

The walls of the primary sporogenous cells are at first no thicker than those of the surrounding tissues but they soon increase in thickness and stand out conspicuously from the neighbouring membranes. The very young anther contains only a trace of starch in fine granules. The occurrence of starch can first be detected in the filament of the stamen, it then spreads upwards to the cells of the connective which lie dorsal to the vascular bundle and it can next be seen in the primary sporogenous cells. This is the usual sequence of starch appearance but the conditions under which the plant has been grown and the time of day when the anthers have been fixed exercise, at all stages of development, considerable influence over the starch-contents of the anther.

Certain broad facts of starch-distribution, however, remain fairly constant in healthy plants grown under average conditions.

In *Gaura* the single longitudinal series of primary sporogenous cells becomes, without any further longitudinal division, the single column of pollen-mother-cells, each of which becomes

[1]) A little starch occasionally occurs in the epidermal cells of the connective just over the vascular bundle, but never in any other part of the epidermis.

surrounded by a mucilaginous wall of peculiar nature. In *Epilobium tetragonum* the primary sporogenous cells undergo a single longitudinal division so that two rows of mother-cells are formed whilst in *Oenothera* a second longitudinal wall often follows the first so that either two or tree mother-cells are seen in the transverse section of each pollen sac. The next important step in development is the formation of a mucilaginous wall round each mother-cell.

This wall is essentially similar to that which occurs in a corresponding position in other angiosperms. Mangin[1]) examined the mother-cell walls of a number of flowering plants and concluded, from their microchemical behaviour, that they consist of callose in a peculiarly pure state. In *Gentiana officinalis* and *Campanula rapunculoides* Mangin[2]) noted some variations in the composition of the (special-) mother-cell wall.

From the facts shat this wall, in the *Onagraceae*, stains deeply with a solution ef corallin in soda (4 % Na_2CO_3), with aniline blue, benzo-purpurin or congo red, that is gives none of the cellulose reactions with Iodine reagents and is insoluble in cuprammonia, and that it has no affinity for ruthenium red. I agree with Mangin in considering callose to be its only constituent.

In several respects, however, I find the reactions of the mother-cell wall to disagree from those usually attributed to callose. Callose is described as readily soluble in 1 % caustic potash or soda. I find this statement to require some modification with regard to the mother-cell wall. The mother-cell wall of fresh material of *Aucuba japonica* dissolved with exemplary rapidity in 1 % caustic soda but I have kept microtome sections of material of *Oenothera* fixed with Flemming's solution for over an hour in 1 % caustic potash and still found the mother-cells undissolved at the end of that time. The mother-cell walls of fresh material of *O. biennis* had only disappeared after nearly 24 hours in 1 % NaOH. I have found fresh material of the pollen-mother-cells of the Horse-chestnut equally resistant to 1 % caustic alkali. In 10 % caustic potash the mother-cell walls of *Oenothera* soon disappear. It will be seen from these remarks that there is some variation in the solubility of the mother-cell wall in dilute caustic alkalis.

Mangin has affirmed that callose is soluble in phosphoric acid but I have left the mother-cell walls of *Oenothera biennis* for many hours in strong phosphoric acid without obtaining any signs of their solution. Naphtol black, in acid solution, is said by Mangin to Stain cellulose but to leave callose uncoloured. I have obtained precisely the opposite result. Bismarck brown,

[1]) Mangin, „Observations sur la membrane du Grain de Pollen mur". (Bull. Soc. Bot. d. France. T. 36. 1889.)

[2]) Mangin, „Observations sur le développement du pollen". (Bull. Soc. Bot. d. France. T. 36. 1889.)

methylene blue and fuchsin are all described as pectic stains which leave callose uncoloured. I have found them all to stain the mother-cell walls although not nearly so deeply as the pectic membranes.

The origen of callose has formed the subject of repeated discussion. In the case of the callose of the Sieve-tubes some have asserted that this substance arises from the transformation of pre-existing cellulose whilst others believe it to be a direct product of protoplasmic activity originating without any relation to cellulose or other fore-runner. Hill in his account of the sieve-tubes of *Pinus*, takes up an intermediate position and believes that callose may originate sometimes directly and sometimes indirectly[1]).

In the case of the callose composing the pollen mother-cell walls there can be no doubt concerning its mode of origen.

It has already been mentioned that in the very young anther the walls of the primary sporogenous cells are poorer in cellulose than the other tissues of the anther. In somewhat older anthers, but still long before the mother-cell wall may he expected to appear, the membranes of the sporogenous cells no longer show any traces of cellulose. Even after treatment with dilute acid and alkali — as recommended by Mangin — I was unable to demonstrate any cellulose in these walls.

It is within these walls that the callose layer is developed. There is, here, no disappearance of either cellulose[2]) or pectose to account for a transformation of these substances into callose. Whatever may be the explanation of the formation of callose in sieve-tubes, I think there can be no doubt that in the case of the pollen-mother-cells the callose is derived directly from the activity of the protoplast without the intermediation of cellulose.

Each mother-cell now divides to form the four special-mother-cells. The mitotic figure is rather small and not well adapted for studying the details of nuclear division.

I will content myself, therefore, with stating that in *Oenothera longiflora* the number of chromosomes which appear at the first and second divisions of the pollen mother-cell is seven. They are so small in size that I can only distinguish them as somewhat irregular granules; whether they have a definite and constant shape peculiar to each division (as seems likely) could not be certainly determined (Figs. 5—9). In the somatic divisions (which I have studied in the wall-cells of the anther) the chromosomes have the form of curved rods which are crowded

[1]) „The Histology of the sieve-tubes of Pinus". (Ann. of Bot. Vol. XV. 1901. p. 597.)

[2]) The cellulose is lost sight of in the walls of the sporogenous cells far too long before the callose appears for these substances to have any connection with one another. Moreover the cellulose which occurs in the young sporogenous wall is merely a trace and could not possibly account for the massive callose wall.

together upon the spindle so that it is not easy to count them. I have distinguished 13 to 14 in some cases and the latter number will probably prove to be the correct one.

Between the cells of the tetrad, which results from this division. Septa are developed which form an extension of the mucilaginous mother-cell wall. Like the latter these septa also give the reactions of callose (Figs. 10 and 11).

Mangin[1]) has called attention to three delicate lines which run through the middle of the septa of the fully grown special-mother-cell wall and join one another at the centre of the tetrad.

He pointed out that these lines were often granular in structure and he believed them to be nitrogenous in nature.

Other authors have figured these radiating lines in the special-mother-cells of other plants; Strasburger figuring them both for *Althaea rosea* and *Gaura biennis* as long ago as 1882.

I have observed these lines in all the *Onagraceae* which I have examined. By careful focussing and by the comparison of series of microtome sections. I find these lines to be the optical expression of laminae. Most probably these laminae represent the first lamellae deposited after the completion of cell division. They differ somewhat from the rest of the special-mother-cell wall in their behaviour towards stains but their reactions still indicate their callose composition (Figs. 12 and 13). Moreover at a later stage, when the special-mother-cell wall breaks down and the pollen grains are liberated, these lamellae remain behind for some time unchanged and continue to give a very characteristic callose reaction with corallin-soda (Figs. 51 and 52).

In anthers which are a little older we observe the first appearance of the pollen membrane round each special-mother-cell.

We first recognise it as a very delicate film lining each cell-cavity of the tetrad. It is in most intimate contact with the callose wall and even reagents which cause general plasmolysis and considerable distortion of the cell-walls of the anther seldom separate the very young pollen membranes from the special-mother-cell wall. The protoplast of the cell is also firmly attached to the new membrane but it is easier to tear away the cytoplasm from it than it is to separate this film from the callose wall. From the first, however, it can he distinguished from the special-mother-cell wall by its behaviour towards reagents. It stains red with ruthenium red; it colours much more deeply than the callose wall with bismarck brown, fuchsin, or methylene blue; it is unstained by corallin soda, and it becomes yellow or brown in chlor-zinc-iodine solution.

[1]) Bull. Soc. Bot. d. France. T. 36. 1889. p. 391. Mangin described this in the special-mother-cell wall of *Althaea rosea*.

When tetrads at this stage are treated with 10% KOH the callose wall is dissolved and the protoplasts, each surrounded by the undissolved pollen membrane, are set free.

We may conclude from these reactions that the young pollen wall is composed of a pectic substance. The remains of the primary sporogenous cell wall, which also gives the reactions of a pectic body, can still he distinguished at the periphery of the tetrad. Although the association between the newly developed pollen membrane and the special-mother-cell wall is so close the demarcation between the two is always sharp and there is never a gradation of one into the other. Where the callose wall abuts upon the pectic membrane it is denser and refracts the light more strongly than the rest of the wall probably forming there a „Grenzhäutchen" in Strasburgers sense.

The facts show that although the pollen wall is at first deposited in close contact with, and probably in actual attachment to, the special-mother-cell wall it is not derived from a transformation of the innermost lamella of this callose wall but is directly secreted as a pectic layer by the cell-protoplast. It is equally certain that the plasmoderma[1]) is not bodily transformed into the pollen wall but that this is deposited upon the surface of the plasmoderma, as Strasburger has shown in other casese.

As soon as the pollen membrane becomes slightly thicker it separates readily from the callose layers and is then clearly recognised as an independent structure. The young pollen grain is a bluntly triangular, basin-shaped structure with the concavity of the „basin" directed towards the centre of the tetrad. It measures about 19 to 20 μ across its broadest surface in *O. biennis*. At the apex of the three angles of the pollen grain the wall is extremely thin. The protoplasm fills the cell-cavity and contains a considerable quantity of starch (Figs. 15 and 16).

In pollen grains which are a little older [mearuring about 22 to 24 μ across in *O. biennis*[2])] the wall has thickened considerably and a mucilaginous material has been developed at the three ungles of the cell at those spots which previously were thin (Fig. 17). This mucilage gives the reactions of a pectic substance and appears to he derived from the growth and physical alteration of the pollen wall at these points. The little plugs or discs of mucilage continue to enlarge and soon bulge so far within the cell that they overlap the unswoln pollen wall on each side.

[1]) I use this term as the equivalent of the german „Hautschicht". The word was suggested for this purpose by Strasburger and first used by Stevens in his paper upon „Gametogenesis and Fertilization in Albugo", (Bot. Gazette XXXII. 1901. p. 92.)

[2]) In stating the size of the pollen grain I have always taken the measurement across the broad face of the grain from the tip of an interstitial body to the outer surface of the wall immediatly opposite.

The name of „Zwischenkörper", introduced by Fritsche, has been used by Nägeli and Strasburger in describing these peculiar mucilaginous discs of the Onagraceous pollen grain. I shall speak of these discs as „interstitial bodies" in the present paper. Three is the normal number of interstitial bodies possessed by the pollen grains of all the species of *Onagraceae* which have been examined. In a few cases, however, I have noticed four or even five of these bodies whilst in others only two or one interstitial body occured (Figs. 21 and 22). An interesting abnormality has been noticed in some pollen grains of this age. Instead of the single nucleus which is normal at this time pollen grains have been seen which contain two nuclei: a large one and a small one (Fig. 18). The case is probably to be compared with the irregularities which Juel[1]) and others have described in the nuclear division of the pollen-mother-cells of *Hemerocallis fulva* and is no doubt due to one or more chromosomes becoming separated from the rest and forming an independent nucleus.

There appears to be some variation in the exact time when the special-mother-cell wall breaks down and sets free the pollen grains. A large number of my preparations of *O. biennis* show this to occur at the comparatively early age that we are now considering (viz pollen 22—24 μ across). As was remarked above the first-formed laminae of the special-mother-cell wall maintain their individuality the longest and continue to give callose reactions for some time (Figs. 51 and 52). The rest of the wall now forms a homogeneous mucilage filling the pollen-sac and occupying all the spaces between the pollen-grains. It no longer has any affinity for corallin-soda and its reactions furnish no clue to its chemical nature.

As the pollen grains continue to develope the interstitial bodies become more prominent towards the exterior, giving the broader face of the grain a more pronounced triangular outline.

A secondary thickening layer is now formed within the first pollen wall.

This layer extends over the whole inner face of the first membrane of the pollen grain. It runs up the sides of each interstitial body as a cylindrical extension which gradually thins off as it approaches the apex of the body and dies away altogether at the summit itself (see Figs. 19 and 23). The microchemical reactions of the thickening layer do not exactly correspond with those which are characteristic of any of the ordinary cell-wall components and its chemical nature must for the present be left an open question. With a rather strong solution of Iodine in potassium iodide it gives a very beautiful violet colour but with chlor-zinc-iodine and with a calcium-chloride solution of Iodine it tinges only yellow or yellow-brown. Congo-

[1]) Juel, O. H., „Die Kernteilungen in den Pollenmutterzellen von *Hemerocallis fulva* etc." (Prings. Jahrb. f. wiss. Bot. XXX. 1897. p. 205.)

red leaves it unstained. Methylene-blue and fuchsin mixture stains the layer pink or violet.

I have found that the first pollen-wall of the Horse-chestnut in its early stages, gives reactions which are almost identical with those of the secondary layer of *Oenothera*. Apart from the very striking violet reaction with the Iodine solution the properties of these membranes correspond fairly well with those characteristic of pectic substances and it is not improbable that we are here dealing with an association between a pectic body and a substance of unknown nature. Additional support is given to this view by the fact that the violet reaction becomes lost after treatment of the pollen grains with absolute alcohol, no doubt because the body which gives this reaction is soluble in alcohol. In its behaviour towards other reagents, however, the thickening layer remains unaltered after an immersion in alcohol. Cuticularisation takes place very early in these membranes[1]) and the violet-reacting body may he associated with the first stages of this process.

In alcohol material the thickening layer, at the early periods of its development, is often greatly swollen[2]) and this becomes more marked and may even lead to the bursting of the pollen grain if this be examined in aqueous solutions.

The interstitial body is now[3]) limited towards the cavity of the grain by a closing disc which has the same composition as the rest of that body although it is somewhat denser. The reactions of the whole interstitial body have undergone a change and are no longer those of a pure pectic body. With Iodine reagents it colours yellow; with congo-red it stains uniformly red; with naphtol black it colours blue-black; with nigrosin it becomes black; with ruthenium red it stains red; with methylene blue-fuchsin mixture it colours blue, pink or violet according to the strength of the solution used; with corallin-soda solution it remains colourless.

The protoplast fills the cavity of the pollen grain at this stage but weak plasmolysing agents show that, whilst it is firmly fixed to the developing secondary layer, it is free from the bases of the interstitial bodies.

As the thickening layer of the pollen wall continues its development ring-shaped ridges make their appearance at the bases of the interstitial bodies. These are at first low and inconspicuous but soon become sharp and prominent features on the membrane (Figs. 23 and 24).

In pollen grains of *Oenothera longiflora*[3]) which measure

[1]) After which they colour yellow to brown with Iodine in potassium iodide solution.

[2]) This was already noticed by Strasburger in *Gaura biennis*.

[3]) Pollen grains measure at this time 35 to 38 μ in *Oenothera biennis* and *O. longiflora*.

[3]) Although I give here the actual description and measurement of the pollen of *Oenothera longiflora* the facts are essentially the same in *O. biennis*.

from about 40 to 45 μ the protoplast still completely fills the cell-cavity but it has become entirely free from its walls.

The further increase in the size of the pollen grain which now takes place is more rapid than that of the living protoplast which consequently no longer fills the cell-cavity (Fig. 24). We have here, in fact, conditions which strikingly recall those which Fitting and others[1–4]) have described in the case of the megaspores of *Isoetes* and *Selaginella*.

These results have such an important bearing upon our conceptions of the growth of vegetable membranes and render some features of this process so difficult to understand that several botanists have hesitated to accept them until they could be placed upon a broader basis them was done by those who have examined the megaspores of the Lycopodiales.

With the exception of Fitting, these authors have exclusively rested their conclusions upon microtome sections. Invaluable as such sections are we must not overlook the fact that the long series of manipulations necessary for killing, fixing and embedding in paraffin introduce many possible sources of error and the results obtained by this means should be carefully checked by observations upon living material.

Fitting worked largely with living spores which he examined partly in physiological salt solution and partly in water.

Unfortunately he gives us no details of his methods and it would be very desirable to know exactly what was the strength of his physiological salt solution and whether this particular concentration was found by direct experiment to produce less change in the cell than any other strength. His selection of water as an alternative medium in which to examine the condition of the protoplast was most unitable as water is known to affect the protoplasm and its osmotic condition.

The pollen grains of *Oenothera* are particularly favourable for investigation and I have attempted to make my examination of them as complete as possible. Fresh material has been examined in the first place and the results thus obtained have been compared with microtome sections of material fixed with strong and weak Flemming's solutions, with strong and medium chrom-acetic solution[5]), Merkel's fluid and Worcester's fluid[6]).

[1]) Fitting, H., „Bau und Entwickelungsgeschichte der Makrosporen von *Isoëtes* und *Selaginella* etc." (Bot. Zeit. Bd. 58. 1900. pp. 107—164.)

[2]) Denke, P., „Sporenentwickelung bei *Selaginella*". (Beihefte z. Bot. Centr. Bd. XII. 1902. p. 182.)

[3]) Lyon, M. F., „A study of the Sporangia and Gametophytes of *Selaginella Apus* and *S. Rupestris*". (Bot. Gazette Vol. XXXII. August-September 1901. pp. 124—141 and pp. 170—194.)

[4]) Campbell, H. D., „Studies on the Gametophyte of *Selaginella*". (Annals of Bot. Vol. XVI. 1902. pp. 419—428.)

[5]) Formulae in Chamberlains „Methods in Plant. Histology" p. 28.

[6]) Formula for this fluid was obtained from H. S. Reeds paper upon enzyme secreting cells of Zea and Phoenix. (Ann. Bot. April 1904. p. 271.)

The microtome sections were particularly useful in showing the exact relations which exist between the pollen grains and the other cells of the anther at the different periods of development.

I will give here a few of the measurements which I have made of the pollen grain, its cell-cavity and its protoplast. The stamens were examined directly after the removal of the flower buds from the plants which were all strong healthy individuals growing upon an open plot of ground. The pollen grains were carefully teased out of the anther into a drop of the fluid which was being studied and rapidly examined whilst still uncovered.

I. The stamens from one bud were successively examined in the following solutions (*O. longiflora*).

1. 0,6 % Na Cl.
   Pollen grain = 42 μ,
   „ cavity = 30 μ,
   „ protoplast = 30 μ.
2. 0,75 % Na Cl.
   Pollen grain = 46 μ,
   „ cavity = 30 μ,
   „ protoplast = 30 μ.
3. 2 % Na Cl.
   This caused complete plasmolycis.
   Pollen grain = 40 μ,
   „ cavity = 26 μ,
   „ protoplast = 18 μ.
4. Egg-white,
   Pollen grain = 40 μ,
   „ cavity = 26 μ,
   „ protoplast = 26 μ,

The results in this reagent were particularly uniform.

5. Strong Flemmings solution.
   a) Pollen grain = 42 μ,
   „ cavity = 28 μ,
   „ protoplast = 26 μ.
   b) Pollen grain = 42 μ,
   „ cavity = 28 μ,
   „ protoplast = 28 μ.
6. Strong chrom acetic solution.
   Gave results similar to the Flemmings solution.
7. Merkels solution.
   Pollen grain = 40 μ,
   „ cavity = 30 μ,
   „ protoplast = 30 μ.

Although difficult to recognise at this stage Merkels solution caused the protoplast to swell up and enlarge somewhat.

II. The stamens from another bud were examined in
1. 0,75 % Na Cl.
   Pollen grain = 62 μ. 68 μ, [1])
   „ cavity = 36 μ. 36 μ,
   „ protoplast = 24 μ. 30 μ.
2. Egg-white.
   Pollen grain = 62 μ. 70 μ,
   „ cavity = 36 μ. 36 μ,
   „ protoplast = 26 μ. 32 μ.
3. Strong Flemmings solution.
   Pollen grain = 64 μ. 70 μ,
   „ cavity = 38 μ. 40 μ,
   „ protoplast = 26 μ. 30 μ.
4. Merkels solution.
   Pollen grain = 66 μ,
   „ cavity = 40 μ,
   „ protoplast = 40 μ.

This caused the protoplast to swell up.

5. Strong chrom-acetic solution.
   Pollen grain = 66 μ,
   „ cavity = 36 μ,
   „ protoplast = 28 μ.

III. Stamens from another bud examined in
1. 0,75 % Na Cl.
   Pollen grain = 80 μ,
   „ cavity = 46 μ,
   „ protoplast = 32 μ.
2. Examined in a drop of juice squeezed from the stem of *Oenothera longiflora*.
   Pollen grain = 76 μ. 76 μ. 80 μ,
   „ cavity = 42 μ. 44 μ. 46 μ,
   „ protoplast = 32 μ. 36 μ. 34 μ.
3. In 5 % cane-sugar solution.
   Pollen grain = 72 μ.
   „ cavity = 42 μ,
   „ protoplast = 30 μ.

IV. In another bud pollen examined in a drop squeezed from stem of *Oenothera longiflora*.
   Pollen grain = 72 μ,
   „ cavity = 42 μ,
   „ protoplast = 34 μ.

V. In another bud pollen was examined in
1. 0,6 % Na Cl.
   Pollen grain = 62 μ,
   „ cavity = 34 μ,
   „ protoplast = 28 μ.

[1]) The successive numbers (62 μ and 68 μ in this case) denote measurements of several pollen grains. Each vertical series of figures corresponds to one pollen grain.

2. Absolute Alcohol.

Pollen grain = 62 μ,
„ cavity = not measured,
„ protoplast = 16 μ.

Alcohol always caused great shrinkage of the protoplast.

VI. The following observations were made upon the pollen grains in the following media.

1. The stamen was placed in a drop of olive oil and the pollen carefully teased out without coming into contact with the air.

Pollen grain = 68 μ.
„ cavity = 41 μ,
„ protoplast = 28 μ.

2. Pollen grains teased rapidly into distilled water and immediatly examined showed a protoplast of 28 μ in pollen grain 68 μ across; very soon however the vacuoles of the protoplast enlarged, ruptured the separating arms and laminae of cytoplasm and ran together so that the protoplast slowly swelled up until it quite filled the pollen-cavity (42 μ).
3. In 2% cane sugar the results were similar to those in distilled water.
4. In 5% cane sugar.

The protoplast for some time maintained its size 28 to 30 μ. Later, however in some of the pollen grains changes similar to those of 2. and 3. were seen but much less marked.

5. In 0,6% Na Cl.

The protoplast measured about 28 μ and it remained unaltered after a prolonged examination.

6. In 2% Na Cl.

Protoplast measured only 20 μ and was obviously plasmolysed.

The following observations upon *O. biennis* may also be mentioned.

I carefully and as rapidly as possible teased out the pollen grains into a little of the mucilaginous fluid which can be squeezed from the anther itself. In a pollen grain measuring 68 μ and with a cavity of about 40 μ the protoplast measured 30 μ.

I then added a drop of 0,6% Na Cl solution to the above, watching the effect all the time. The pollen grains remained unchanged both in their appearance and in their measurements.

In another similar experiment upon another bud from a different plant the measurement both in the anther-juice and in the 0,6% Na Cl solution were:

Pollen grain = 74 μ,
„ cavity = 40 μ,
„ protoplast = 30 μ.

I might occupy many pages in quoting similar measurements but as those which I have already mentioned are quite typical of the rest it would serve no useful purpose to do so. The general result has been to show that in 0,6% Na Cl, 0,75% Na Cl, 6% cane-sugar, egg-white and the plants own juice the protoplast has a very similar appearance and its measurements agree very well with one another at the different stages.

Moreover, after remaining in these solutions for some time little or no alteration, either in size or appearance, was observable. The average measurement in these solutions calculated from all my notes are as follows;

Pollen grain	pollen cavity	pollen protoplast
40 μ	26 μ	26 μ
62 μ	37 μ	27 μ
70 μ	39 μ	32 μ
74 μ	42 μ	34.25 μ
80 μ	46 μ	34.5 μ.

Strong Flemmings solution and strong chrom-acetic solution do not alter the protoplast very much in appearance but usually cause some shrinkage.

Merkel's fluid was not so satisfactory and it causes the vacuoles to swell up and the protoplast to enlarge.

Absolute alcohol causes very considerable contraction of the protoplast.

Distilled water enlarges the vacuoles and causes them to run together by breaking down the separating arms and laminae of cytoplasm. Consequently the whole protoplast swells up greatly.

Objection may still be taken to conclusions drawn from a study of the living pollen in the plants own juice, the salt solutions and in egg-white, on account of the possible influence which the mechanical operation of teasing out the pollen grains may have exerted.

That mechanical disturbances can affect the living contents of these cells is shown by the fact that if the pollen grains, in e. g. 0,6% Na Cl solution, are covered by a cover glass and the pressure due to this is not relieved the protoplast gradually enlarges and may finally fill the cell-cavity. If, however, the precaution be taken, of preventing the pressure of the cover glass, by a fragment of anther or filter paper or by not covering the preparation at all no such change takes place in the protoplast.

An error from this cause, however, is extremely improbable as the pollen grains can be drawn out from the anther without actually subjecting them to the touch of an instrument and with only very little pressure or friction. This can be done by means of the fibrous mucilage which surrounds the pollen grains and binds them together in long strings.

Moreover microtome sections of pollen grains, fixed whilst lying untouched within the anther, show a rough parallel in

their measurements with those described for fresh material. I will not however, lay great weight on the evidence of the microtome sections as, in spite of every precaution, I never succeeded in entirely avoiding shrinkage of the pollen-protoplast even when all the other cells of the anther were un-contracted. I will add here a comparison between the measurements of the pollen from a living anther with those of microtome sections:

1. Fresh material of *Oenothera biennis* examined in the juice squeezed from the anther:
   Pollen grain = 74 μ,
   „ cavity = 40 μ,
   „ protoplast = 30 μ.
2. Sections of anther of about same age fixed in Flemmings solution:
   Pollen grain = 72 μ . 70 μ . 70 μ . 70 μ,
   „ cavity = 42 μ . 40 μ . 40 μ . 40 μ,
   „ protoplast = 26 μ . 28 μ . 24 μ . 22 μ.

During the whole time that the protoplast is separated from the membrane in this way the latter continues to grow both in extent and in thickness. We are at present quite in the dark regarding the manner in which this growth takes place but a very brief theoretical consideration of the subject will be found among the conlusions at the end of this paper.

We must now enquire whence is derived the material necessary for this growth.

There are two sources from which the plastic material of the membrane might be derived, viz the protoplast of the pollen grain itself or the tapetum.

That metabolic processes of no mean order are taking place in the former is evident from a study of the changes which can be observed in it during this period.

Starch appears and disappears in the pollen grain in a manner which shows that carbohydrates are being used up in the cell; the cytoplasm continually grows less and less in amount whilst a liquid, apparently the direct consequence of the foregoing processes, gradually forms in the protoplasm.

This liquid first occupies small vacuoles in the cytoplasm, these continue to increase in size and run together (Fig. 24) until we find nothing left of the protoplasm but a hollow shell consisting of a plasmoderma (Hautschicht) and a nucleus, surrounded by a trace of granular cytoplasm (see Fig. 25 which gives a rather later stage). The centre of the shell is occupied by one enormous vacuole[1]).

There is no reason to doubt that this liquid diffuses out from the protoplast into the space which is forming between

[1]) Strasburger in his work of 1882 already wrote of *Gaura biennis*. „In meinen Alkoholpräparaten bildet der nach Anlage der Wand erschöpfte Inhalt der Pollenzelle nur noch ein unscheinbares Klümpchen" cf. his Figs. 47, 48 and 49, Tafel VI.

itself and the pollen-wall and, in all probability, the latter derives the necessary materials for its growth from this source.

Unfortunately I could gain no knownledge whatsoever of the chemical nature of this liquid.

In the tapetum we can also observe evidences of metabolic activity but I can find nothing to show that any of the material which is being formed there is leaving the cells, on the contrary there is reason to believe that an accumulation of substance is taking place.

In the very young pollen grain[1]) the first wall appears as a single homogeneous lamella but when the grain has grown and measures about 40 μ across we can indistinctly recognise a structural differentiation in the outer membrane.

When the diameter of the pollen grain has increased still further (to about 55 to 60 μ) its first membrane can be clearly seen to consist of a thin, outer homogeneous layer and an inner "rodlet" layer (Stäbchenschicht or Anschlußlamella[2]).

The growth of this membrane recalls Strasburger's[3]) description of the first pollen-wall of *Althaea rosea* which, at a certain stage, was seen to consist of three lamellae: a middle "rodlet" layer (Anschlußlamella) which is bounded peripherally by two homogeneous layers. Of these the innermost lamella soon ceases to grow and becomes gradually more attenuated until it is lost sight of altogether; the two other lamella increase in thickness and the "rodlets" can be very clearly studied in older stages. A thickening layer is developed within the first pollen membrane of *Althaea*.

In *Oenothera* the first wall is so thin during its early development that I have not been able to determine whether the "rodlet" layer is ever bounded internally by an inner homogeneous lamella; it is certain, however, that by the time the pollen grain has reached 55 to 60 μ in diameter every trace of it has vanished.

The first pollen-wall now grows more rapidly in surface than the secondary thickening layer beneath it and consequently it becomes separated from that layer at all parts and only remains firmly fixed to the interstitial bodies. The continuation of this unequal growth in surface gradually throws the outer wall into irregular and sinuous folds. Both primary and secondary layers of the wall have meanwhile undergone a change in their chemical constitution and have become more or less completely cuticularised. The secondary layer no longer gives a pure violet colour with a solution of Iodine in potassium iodide but this has changed first to a violet-brown and then to a pure brown reaction.

[1]) This description of the pollen-wall applies both to *Oenoth. longiflora* and to *Oen. biennis* unless specially stated to the contrary. The measurements more particulary refer to *O. biennis* but the dimensions are only very slightly, different in *O. longiflora*.

[2]) Strasburger, "Die pflanzlichen Zellhäute". (Pringsh. Jahrb. f. wiss Bot. Bd. XXXI. 1898. p. 551.)

[3]) l. c. p. 555.

The interstitial bodies are shut off from the cavity of the pollen grain by a well developed closing-disc which consists of two parts; an outer dense and homogeneous layer and an inner less dense, stratified lamina which is cap-shaped in form with the concavity directed towards the cell cavity. Lying on the outer lamina of the closing-disc is an aggregation of granular material which extends some little way up the sides of the interstitial body. The cavity of the interstitial body is no longer occupied by the mucilaginous deeply staining substance which filled it at an earlier period, but it now contains a watery fluid which does not readily stain. I believe that the mucilaginous material of the young interstitial body has been, to a great extent, used up in forming the closing-disc and that the granular substance which lies upon the outer portion of the disc is a remnant of the stainable material. (See Fig. 23.)

In pollen grains which measure between 85 μ and 95 μ in both *O. biennis* and *O. longiflora* the protoplast has become reduced to a hollow sphere or vesicle which has expanded again until it is nearly or quite in contact with the cell-wall (Fig. 25). At one point upon the protoplasmic vesicle a flattened, rather dense nucleus can be seen which encloses a nucleolus. A little finely granular cytoplasm surrounds the nucleus but in its other parts the protoplast appears to be reduced to a plasmoderma (Hautschicht) which surrounds the enormous central vacuole. Very soon the nucleus enlarges, becomes rounder and less dense and passes into the prophases of mitotic division (Fig. 26). I have not followed the details of this division which leads to the formation of two distinct cells within the pollen grain: the large vegetative cell and the small generative cell. The latter is limited by a well marked plasmoderma (Hautschicht) (Fig. 27).

The tapetum now breaks down and its contents clearly furnish the material for the renewed growth of the pollen-protoplasts.

In order to understand the nature of this material it is necessary to consider the changes which take place in the tapetum during its earlier development.

In the very young anthers, before the full number of primary sporogenous cells is established, the tapetal cells contain a not very dense cytoplasm which encloses a single nucleus.

This nucleus, besides small, scattered chromatin granules, contains one to four nucleoli.

The nuclear membrane colours deeply with iron-haematoxylin or with methylene blue-fuchsin mixture. Very rapidly the cytoplasm increases in density and the originally single nucleus divides into several, as many as eight nuclei being not uncommonly met with in a cell (Fig. 30 and 31).

Until about the pollen-mother-cell stage the tapetal nuclei multiply exclusively by mitotic division but at the mother-cell stage nuclear figures occur which are strongly suggestive of

fragmentation. When the special-mother-cells have been established mitotic divisions are rarely met with whilst fragmenting nuclei occur on every side. Most of the tapetal nuclei now contain a single large nucleolus and a very deeply staining nuclear wall, besides this only a very little finely granular chromatic material can be seen lying near or upon the nuclear wall (see *n* Fig. 44).

In anthers in which the first pollen-wall is just making its appearance. I have several times seen the tapetal nuclei in the prophases of mitosis but I have never, at this period, succeeded in finding the later stages of division and I believe that mitosis is no longer completed by the nuclei. Nuclei which have every appearance of undergoing fragmentation are, however, very abundant both at this and at later stages of development (Fig. 32, 33, 34, 40, 42) Strasburger[1]) and later writers, in describing the tapetum of other plants, have found mitosis to be the only mode of nuclear division and they believe the constricted nuclei which occur in the cells to represent fusion and not fragmentation of the nuclei.

In *Oenothera* it is impossible to imagine that karyokinesis can be the only mode of nuclear multiplication.

In the first place mitotic divisions are never very frequent and it is difficult to account for the presence of six or seven nuclei in a young tapetal cell through their agency alone.

Moreover, mitotic figures cannot be found in the tapetum of *Oenothera* after the appearance of the first pollen wall so that if this is the only mode of division and the constricted nuclei, which are common both at this and at subsequent stages, really represent fusions it is impossible to see whence the constant supply of nuclei comes for these repeated fusions and which leaves the older tapetal cell with two or three nuclei to the last. The way in which these constricted nuclei often hang together by a narrow neck also favours the view that they are separating from one another and are not uniting (see especially Fig. 34).

The great disparity which after exists in the sizes of the nuclei of a cell is also what one would expect with direct rather than with indirect division (compare sizes of the two nuclei in Fig. 37).

For all of these reasons I consider that most of these constricted nuclei represent a fragmentation and not a fusion of nuclei.

Every constricted nucleus does not, however, necessarily imply nuclear multiplication.

There is no doubt that the tapetal nuclei alter their shape and often become very irregular in outline without this leading to a division of the nucleus or representing a fusion (see Fig. 36, 37). These changes in shape are evidently signs of the occurrence of an active metabolism in the cell and may be compared to the similar phenomena which have been described in the secreting cells of may animals.

[1]) Strasburger, E. „Teilungsvorgang d. Zellkerne etc." (Arch. f. Mikro. Anat. Bd. 21. 1882. pp. 574—575.)

This continuous nuclear multiplication, by both direct and indirect division, must lead to the accumulation of a large number of nuclei in each cell unless an opposite process, reducing their number takes place at the same time.

An glance at a section of an older anther will at once show that no excessive accumulation of nuclei occurs in the cell and I have succeeded in finding clear evidence of a nuclear degeneration taking place side by side with the nuclear multiplication.

In this process the nuclear membrane, which stains very deeply, becomes ruptured and shredded out into a group of fibres or narrow laminae whilst the nucleolus can also, in many instances, be seen to resolve itself into a coarse fibre.

There can be little doubt that the groups of fibres formed in this manner correspond to the structures which Meves[1] has recently described in the tapetal cells of *Nymphaea alba* and which be has compared to the chondromiten of certain animal cells.

It is quite easy, in well fixed material,[2] to find all stages between a complete nucleus and one that is only represented by a group of fibres. In Fig. 43, 44 and 45 *d* and *f* I have drawn nuclei which are degenerating in this way.

These fibres, of nuclear origen, become gradually more numerous in older anthers, as the tapetal nuclei continue to divide and to degenerate, but whether they all persist as fibres or whether some of them are lost sight of in the course of further changes I am unable to say. It is certain, however, that the cytoplasm of the tapetal cells which are approaching disintegration stains very deeply and that it contains a large number of these fibres.

Just before tapetal disintegration the whole contents of the cell, apart from the unaltered nuclei,[3] break down into coarse granules which stain intensely with iron-haematoxylin and these become distributed among the pollen grains when the cell loses its individuality.

During the development of the anther starch appears and again disappears in the tapetum according to the conditions of growth and this shows that carbohydrates are being employed in metabolism.

The conclusion which may be drawn from the above facts is that a large part of the material which accumulates in the tapetal cells during their development and which subsequently passes into the pollen grains to replenish their exhausted protoplasts has at one time or another entered into the composition of the tapetal nuclei und that there is here, therefore, a direct relation between nuclear substance and cytoplasmic growth.

[1]) Meves, Fr. „Über das Vorkommen von Mitochondrien bezw. Chondromiten in Pflanzenzellen." (Berichte d. Deutsch. bot. Gesell. XXII 1904. pp. 284—286.)

[2]) Wor cester's fluid is by far the best fixatine for this purpose.

[3]) Which are two or three in number.

The comparison which Meves has drawn between these deeply staining fibres of the tapetal cytoplasm and the chondromiten of certain animal cells is of the highest interest.

In a large number of actively functional cells, belonging to the most various tissues of the animal body, chromatic structures have been found in the cytoplasm und described under the names of mitochondrien, chondromiten, pseudochromosomes, yolk-nuclei, chromidien, apparato reticolare etc.

Goldschmidt[1]) has recently found good grounds for grouping all these structures together and he has shown by direct experiment that at least in some cases (e. g. muscle-cells of *Ascaris lumbricoides*) they are directly connected with the functional activity of the cell.

In the tapetum the fibres (or their derivatives) unquestionably play a prominent part in the cytoplasmic growth of the pollen-protoplasts and no doubt in the animal cell they are also in some way associated with the elaboration of complex organic substances.[2]) In this relation it may be recalled that several physiological chemists have pointed out the probability of nuclein or one of its constituent molecular groups forming a centre or starting point for the synthesis of complex organic matters in the living cell.

The origen and chemical nature of these chromidial structures has, however, not yet been satisfactorily determined in all cases. In some cells which have been studied by Goldschmidt it is highly probable that they are derived from the chromatin of the nucleus.

I have shown above that the fibres in the tapetal cells of *Oenothera* possess a nuclear origen and may be referred to the transformation of the nucleoli and nuclear membranes.

The staining reactions and the behaviour of these nucleoli, whilst the nucleus is still intact, show that they are, at least partly, composed of chromatin whilst the nuclear wall also seems to owe its affinity for nuclear dyes to the deposition of finely granular chromatin upon its inner face or within its substance.

We see therefore that the fibres lying in the tapetal cytoplasm are to a great extent derived from the chromatin of the nucleus and that much of the substance that ultimately passes into the pollen grains is a derivative of chromatin.

The walls of the tapetum, during the greater part of the development of the anther are of a somewhat mucilaginous nature and can be very distinctly differentiated by means of an alkaline solution of congo red. In the older anther these

[1]) Goldschmidt, R. „Der Chromidialapparat lebhaft funktionierender Gewebzellen". (Zoolog. Jahrb. Abt. f. Anat. u. Ontogenie d. Tiere. Bd. XXI. 1904. p. p. 1—100.)

[2]) For example note the relation which Mathews found to exist between the deeply staining fibres and the Zymogen granules of certain pancreas and liver cells. (Journ. Morphol. XV. Suppl. 1899.)

walls become very thin and at the time when the tapetum disintegrates they become so attenuated that at some spots they are apparently interrupted altogether. It is obvious therefore that the tapetal walls offer no great hinderance to the passage of the cell-contents. It is more difficult, however, to understand how the tapetal substance passes through the thick, cuticularised pollen-wall to reach the protoplast. It must evidently do so in a state of solution but how the complex material of the tapetal cells is brought into solution can at present only he guessed at. Enzymes are probably the effective agents but we at present have no knowledge either of their source or nature.

We left the pollen grain at a stage when the protoplast, in the form of a hollow shell, had enlarged sufficiently to fill the cell-cavity once again. At a slightly later period the generative cell and the vegetative nucleus leave their peripheral position for one in the centre of the cell cavity where they are suspended, together with more or less cytoplasm, by three thick strands of cytoplasm and ofter several smaller ones as well. The three thick arms of protoplasm extend to the bases of the three interstitial bodies and it is a significant fact that the intine can first be recognised at these spots and that it here attains its greatest thickness (Fig. 28 and 29). It is difficult to avoid the conclusion that influences of some kind originate in the nucleus and pass along the three arms of cytoplasm to those spots at which new cell-wall lamellae are forming but we are at present quite in the dark as to the nature of these influences.

In still older pollen-grains, measuring from 108 to 112 μ in diameter, the intine forms a continuous layer over the whole inner face of the wall. It is thick and easily seen at the base of each interstitial body but it is extremely delicate elsewhere and can only be traced as a continuous membrane with some difficulty.

The intine gives very clearly the characteristic reactions of a pectic substance but I was not able to demonstrate the presence of cellulose in it with any certainly.

The interstitial bodies contain one or more yellowish globules which usually entangle an air-bubble in them. These globules appear to be of an oily nature for they are blackened by osmic acid and they are soluble in absolute alcohol.

The protoplasm, covered by the intine, now bores its way through the closing disc and enters the interstitial body which it soon entirely fills. I have followed this process most completely in the case of *Gaura Lindheimeri* and I will, therefore, refer to this plant in the present description.

In the quite young pollen grain of *Gaura* the interstitial bodies are composed of a homogeneous mucilage which in every way resembles that of *Oenothera* at a corresponding age.

In older grains this structureless mucilage becomes distinctly laminated. These laminae are very closely arranged at the base of the interstitial body and form there a closing disc.[1])

Above the closing disc the laminae are much more loosely placed and they often become drawn out and even broken at their middle by the growth of the interstitial body. At the apex of the interstitial body the laminae again are very densely arranged.

The intine forms quite a thick pad under each interstitial body but is very thin over the rest of the pollen grain. It contains both cellulose and a pectic body in its composition. Both substances are distributed equally through the thickness of the membrane and there is no differentiation of a pectic layer from a cellulose one.

When the intine and protoplast are about to penetrate the interstitial body we first find that a narrow cleft is bored through the middle of the closing disc (Fig. 46). Then a small fold of intine can be seen to pass into this slit (Fig. 47) and to gradually make its way to the centre of the interstitial body where the laminae are thin or quite broken through. Here it bulges out into a small, thick-walled sac (Fig. 48 and 49). The laminae of the interstitial body are gradually eaten away and the intine-sac continues to grow until it lies closely against the short teeth which alone remain of the interstitial laminae.

It is interesting to note that the intine must be of a very soft and even mucilaginous nature as it often moulds itself to all the irregularities on the wall and "flows" between the teeth which project from the interstitial wall. The opening in the closing disc gradually enlarges until the disc is reduced to a narrow and dense collar or ring (Fig. 50).

The manner in which the closing disc is perforated and the substance of the interstitial body slowly eaten away suggests the presence of a solvent, probably an enzyme, which is secreted by the protoplast and which carries out the work of disintegration. It is difficult otherwise to explain the appearance of a clean cut aperture in the closing disc before the intine grows out to force itself a way. Moreover, the slow dissolution of the interstitial laminae takes place before the intine comes into contact with them so that they cannot be mechanically broken down by the growth of that membrane.

The mature pollen grain of *Oenothera longiflora* measures between 170 and 180 μ across; it is quite filled by the protoplast which is densely crowded with starch. The two layers of the exine are again in contact with one another.

The outer layer is, however, only firmly attached over the interstitial bodies; it consists of an outer, homogeneous lamella which is continuous over the whole pollen grain and the inner

[1]) So closely are the laminae arranged in the closing disc that the laminated appearance is often lost sight of altogether and the disc appears granular.

"rodlet" layer which is interrupted over the apices of the interstitial bodies.

During the later growth of the pollen grain the secondary thickening layer has not increased in thickness but has, on the contrary, become stretched and very much thinner than it was at an earlier stage. (In Fig. 50 the secondary thickening layer has been drawn too thick.)

In *Oenothera longiflora* all the pollen grains do not reach maturity but a large proportion of them become arrested in their development. They all grow to about 90 μ in diameter, when their protoplast has become reduced to a hollow shell, but after that many of them are unable to continue their development owing, no doubt, to the tapetal substance being insufficient for the requirements of all the pollen grains.

I have not given any special attention to the germination of the pollen grain but I may mention that the intine of *Epilobium tetragonum* which gives the reactions of both cellulose and pectose, grows out into a tube which is often branched at its free end (Fig. 20).

The mature pollen grains of *Oenothera* are bound together in long strings by bundles of "fibrils" which lie between and round them. These fibrils are developed from the mucilage which, on an earlier page, we saw was derived from the disintegration of the special-mother-cell walls. These „fibrils" have entirely lost all affinity for callose dyes and have become very resistant to solvents. Their properties in many respects resemble those of cuticularised structures.

In the species of *Epilobium* short bands of the cuticularised mucilage bind together the pollen grains which, consequently, leave the anther in tetrads.

Summary and conclusions.

1. In the earliest stages of anther development all the cell-membranes contain both cellulose and pectose. The walls of the sporogenous cells, however, contain less cellulose than the other membranes of the anther. In older anthers the sporogenous cell-membranes give the reactions of a pectic substance alone.

2. The pollen-mother-cell wall consists of pure callose. This substance is formed directly as such by the protoplast and there can be no possibility, in the present case, of a transformation of cellulose into callose.

3. In the first and second divisions of the pollen-mother-cell seven chromosomes occur whilst in the somatic divisions fourteen is the approximate number of chromosomes. The presence of two nuclei, one large and the other very small, in some quite young pollen grains suggests the occurrence of irregularities in the divisions similar to those described by Juel in *Hemerocallis fulva*.

4. The first pollen membrane is formed by the direct activity of the protoplast and is deposited as a delicate layer of pectic material upon the inner face of the special-mother-cell wall. Although it originates in the most intimate contact with the callose wall it is chemically distinct from this from the very first.

5. The interstitial bodies originate as specialised areas on the first pollen wall. These spots are at first characterised by their greater thinness; later a homogeneous mucilage is developed at these places. In older pollen grains a portion of this mucilage is deposited as a dense closing disc whilst, in *Oenothera*, the rest of the interstitial body is filled with a thin fluid. In *Gaura* more or less solid laminae are deposited throughout the interstitial body.

6. A secondary thickening layer is laid down by the protoplast within the first pollen membrane. This layer gives most of the pectic reactions but also a very distinct violet colour with a strong solution of Iodine in potassium iodide. It gives none of the usual cellulose reactions.

7. Both the first pollen wall and the secondary thickening layer are firmly attached to the protoplast when they are first developed.

In pollen grains which have reached 40 μ in size the protoplast is no longer fixed to the wall at any place although it still completely fills the cell cavity.

The pollen grain continues to grow and its walls increase both in thickness and in extent. Whilst the pollen grain doubles its diameter the cell-cavity increases in size from about 26 μ to about 46 μ. The protoplast, however, grows far less rapidly during this time and its diameter only enlarges from 26 μ to about 34 μ.

In consequence of this inequality in growth the protoplast becomes separated from the pollen wall by a space which is filled with liquid.

The conditions seem to be quite similar to those which Fitting and others have described in the megaspores of *Isoetes* and *Selaginella*.

In *Oenothera* also, as in the megaspores, the growth of the layers of the wall is not equally rapid and the first pollen wall becomes separated from the secondary thickening layer and is thrown into folds upon its surface.

These observations show that during the period of most active growth of the membrane (both in surface and in thickness) the protoplast is completely separated from it and we must conclude either that the growth of a cell-wall is a purely physical process or that the living protoplast can exert its influence across a space filled with liquid.

I may add here that, although the growth of a membrane whilst this is separated from the protoplast by an actual space

has only been found to occur in the few isolated cases mentioned above, other less extreme instances belonging to the same category of phenomena are not unknown. Wherever we find that a new lamella is interpolated between the protoplast and an older lamella and the latter still continues to grow in thickness or in surface it does so whilst it is neither in union or in contact with the living element of the cell.

Meanwhile changes are taking place in the protoplast and we find that a fluid is forming in the cytoplasm, partly at the expense of carbohydrates which have reached it fromwithout and partly at the expense of the cytoplasm itself. This fluid is formed in vacuoles which gradually run together until the protoplast is reduced to a hollow sphere enclosing a single, large, central vacuole. We have reason to believe that this fluid diffuses out from the protoplast and furnishes material for the growth of the pollen-membrane.

The three most important features in the formation and development of the layers of the pollen wall may, therefore, be summarised as follows:

I. Both the primary pollen wall and the secondary thickening layer originate in intimate connection with the plasmoderma (Hautschicht).

II. The greater portion of the subsequent growth of both these membranes takes place by intussusception whilst they are completely separated from the protoplast.

III. The material required for the growth of the membranes is derived from the tecretory activity of the pollen-protoplast.

We can at present only vaguely guess at the most probable way in which the growth of these membranes takes place.

There are some facts, such as Ambronn's work[1]) upon the optical properties of the cuticulariced walls, which indicate that the cell-wall may be underlaid by a crystalline structure and it is possible that when the membrane is first formed the protoplast (to which it is then firmly fixed) determines the character and the arrangement of these crystals.

The later growth of the membrane, even after it has become separated from the living element of the cell, may be considered to take place in a manner which depends upon the nature and relative positions of its crystalline components.

8. After the pollen protoplast has become almost completely exhausted by its secretory activity its substance is once more repenished by the material derived from the disintegration of the tapetum.

The very young tapetum contains a rather scanty cytoplasm and only a single nucleus. Later the tapetal cells are furnished with a denser cytoplasm and nuclei which may vary in number from one to eight. Until the end of the special-mother-cell

[1]) Ambronn, H. Ber. d. Deutsch. bot. Gesell. 1888. p. 226.

stage both mitotic and amitotic divisions of the nuclei take place but in older anthers the tapetal nuclei divide exclusively by amitosis. Side by side with this continuous multiplication of nuclei a nuclear degeneration can be observed in which the nuclear membrane and nucleolus are resolved into a group of deeply staining fibres or narrow laminae.

These fibres are no doubt identical with those observed by Meves in the tapetum of *Nymphaea alba*. These fibres have been compared by Meves to the chromidial structures found in certain animal cells and this comparison has a great interest in the light of Goldschmidt's recent work.

The fibres in the tapetum of *Oenothera* are found to be to a large extent derivatives of the chromatin of the nucleus and they increase in number as the cell advances in age and its nuclei continue to divide (fragment) and to break down.

The interesting conclusion is, therefore, reached that a large portion of the material which replenishes the exhausted pollen-protoplast has at one time or another entered into the composition of a tapetal nucleus.

9. The intine is a continuous membrane lining the entire inner surface of the pollen grain. It first appears and reaches its greatest development at the bases of the three interstitial bodies whilst over the rest of the pollen grain it extends as an exceedingly delicate layer. During the development of the intine three thick strands of cytoplasm connect the centrally placed nucleus with the spots beneath the interstitial bodies where the membrane is growing most vigoously. In *Oenothera* the intine gives the reactions of a pectic body but little or no cellulose can be detected in it. In *Gaura Lindheimeri* and *Epilobium tetragonum* both pectic bodies and cellulose occur in the intine.

10. The perforation of the closing disc of the interstitial body and the disintegration of the laminae of that body have been most closely followed in the pollen grains of *Gaura Lindheimeri*. The closing disc is perforated and the interstitial laminae are "eaten away" in advance of the growing intine in a manner which suggests the action of a solvent, probably an enzyme.

11. In *Oenothera longiflora*, even when growing under the most favourable conditions, many of the pollen grains become arrested in their development. They all seem to advance until their protoplast is completely exhausted by secretion but the tapetal material is insufficient to allow all of them to carry their development further.

12. The mature pollen grains are surrounded and bound together by "fibrils" which are derived from the special-mother-cell wall. When the special-mother-cell wall breaks down it forms at first a structureless mucilage which no longer gives any of the reactions of callose. Later this mucilage becomes drawn out into "fibrils" and these are very resistant to solvents.

Rudolf Beer.

Explanation of Plates 3, 4 and 5.

Fig. 1. Lateral intercellular passage of anther of *O. biennis* in which the walls bordering upon the space have become thickened and cuticularised. Anther at stage of special-mother-cells.

Fig. 2, 3 and 4. Successive stages in the development of stoma upon anther of *O. longiflora*.

Fig. 5—7. Division of pollen-mother-cell showing seven chromosomes in *O. longiflora*.

Fig. 8 and 9. Later stage of division of same. All material from which Fig. 5—9 were drawn was fixed with medium chrom-acetic mixture.

Fig. 10 and 11. Special-mother-cells soon after completion of division. *Oenothera biennis*.

Fig. 12 and 13. Special-mother-cells later stage to show differentiation of the first-formed septa from the later-formed layers of the wall. Fig. 12 examined in chlor-zinc-iodine solution to which a little phosphoric-iodine solution had been added. Fig. 13 examined in corallin soda solution *O. biennis*.

Fig. 14. Raphide-sac of anther of *O. biennis*. Strong Flemming's sol. and Heidenhain's haematoxylin.

Fig. 15 and 16. Young pollen grain of *O. biennis* immediatly after formation of first pollen-wall strong Flemming's sol.

Fig. 17. Slightly older pollen-grain of *O. biennis*. Interstitial bodies formed.

Fig. 18. Similar pollen-grain to that in Fig. 17 but with two nuclei.

Fig. 19. Pollen grain of *O. biennis* measuring 40 μ in diameter. Living material examined in .6% Na Cl solution.

Fig. 20. Branched end of pollen-tube of *Epilobium tetragonum*.

Fig. 21 and 22. Abnormal pollen grains of *O. biennis* with two and one interstitial body respectivly. Examined in .6% Na Cl solution.

Fig. 23. Pollen grain of *O. biennis* 45 μ across Flemming's solution methylene blue and fuchsin.

Fig. 24. Pollen grain *O. longiflora* 70 μ across. Living material examined in .75% Na Cl solution.

Fig. 25. Section of pollen grain of *O. biennis* measuring about 86 μ across the entire grain to show protoplast at end of the period of secretory activity and when it has expanded until it nearly fills the cell-cavity Flemming's sol. Heidenhain's haematoxylin and bismarck brown.

Fig. 26. Similar pollen grain to Fig. 25 nucleus showing first stage of mitosis.

Fig. 27. Section of pollen grain of *O. biennis* measuring about 86 μ across showing vegetative and generative (g) cells. Flemming's sol. Heidenhein's haematoxylin and bismarck brown.

Fig. 28. Pollen grain of *O. biennis* measuring 150 μ across to show the three arms of protoplasm extending to the bases of the three interstitial bodies where the intine was just beginning to be formed. Living material examined in .6% Na Cl solution.

Fig. 29. Pollen grain *O. longiflora* to show the three arms of cytoplasm. Fixed with Worcester's fluid. Methylene blue and fuchsin stain.

Fig. 30. Tapetal cell of *O. longiflora* with 8 nuclei. Strong Flemming sol. Methylene blue and fuchsin preceded by crocein stain.

Fig. 31. Tapetal cell of *O. longiflora* with 6 nuclei.

Fig. 32—34. Tapetal cells of *O. biennis* to show direct division of nucleus. Strong Flemming's solution.

Fig. 35—39. Tapetal cells to show constricted and irregular nuclei some of which may be stages of direct division to others no such significance attaches.

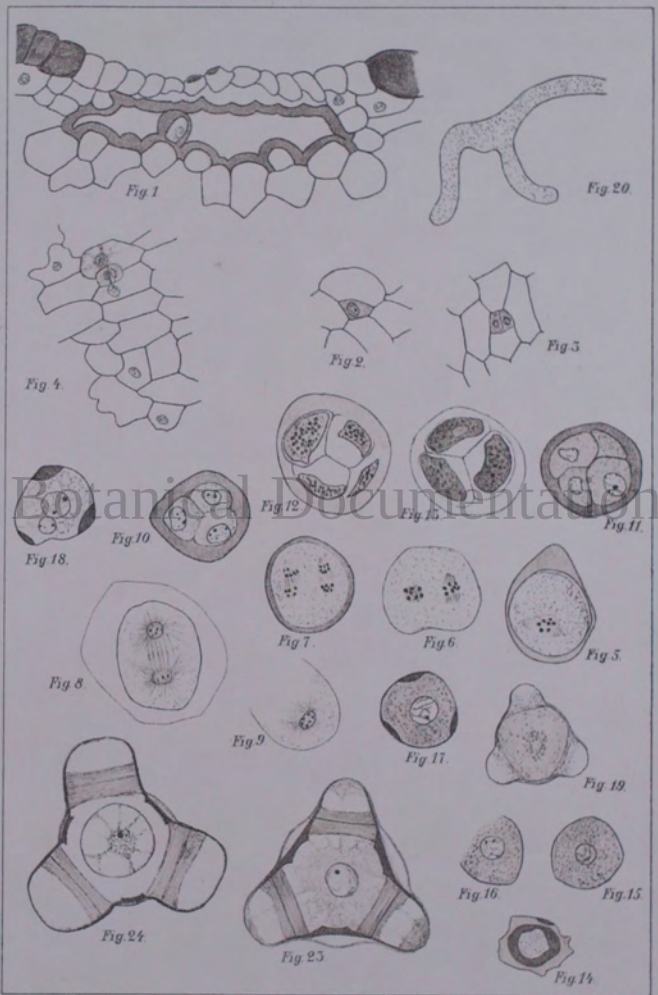
Fig. 40—42. Tapetal cells from somewhat older anthers in which pollen grains measure from 60 to 95 μ across. To show constricted nuclei.

Fig. 43—45. Tapetal cells of *O. longiflora* to show chromidial fibres (f). degenerating nuclei (d) and intact nuclei (n). Worcester's fluid; methylene blue and fuchsin.

Fig. 46—49. Interstitial bodies of pollen grain of *Gaura Lindheimeri* showing successive stages in its perforation and dissolution by the advancing intine Fig. 46—48 examined in chlor-zinc-iodine solution Fig. 49 stained with bismarck brown. All fixed with Flemming's solution.

Fig. 50. Interstitial body of mature pollen grain of *O. longiflora*. Medium chrom-acetic. Bismarck brown.

Fig. 51 and 52. Disintegration of special-mother-cell-wall. *Oen. biennis* strong Flemming. To show the first-formed lamellae of the wall still left intact whilst the later-formed layers have completely, broken down. Fig. 51 congo red stain. Fig. 52 corallin soda.



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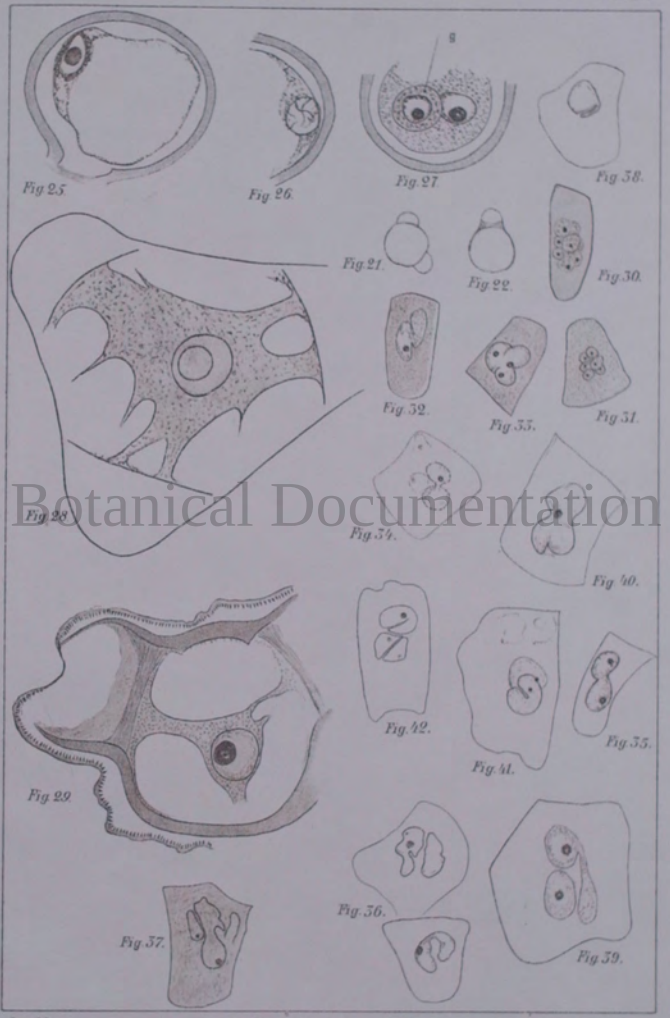

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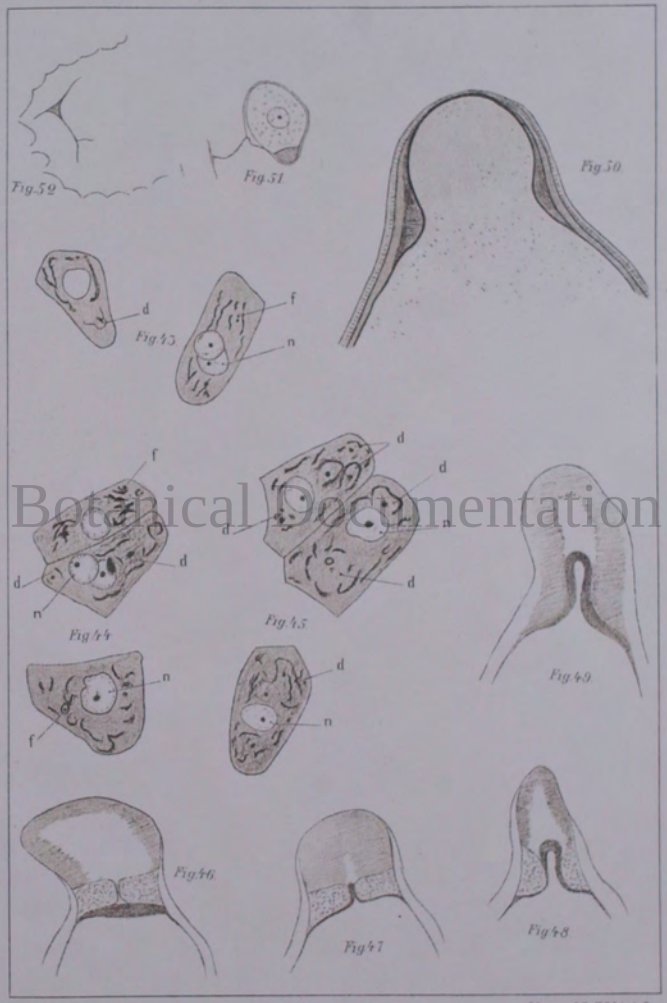

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On the development of the pollen-grain & anther of some Onagraceae

By

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Mit 3 Tafeln

The striking character of the pollen grains of the Onagraceae has attracted the attention of botanists from a very early date.

Already in 1830 Purkinje examined & figured the pollen of several species ("De cellulis Antherarum Fibrosis etc" Vratislaviae 1830) & since that time Hugo von Mohl, Fritsche, Schacht, Nägeli, Luerssen, Tschistiakoff, Sachs, Wille & Strasburger, as well as others have all paid greater or less attention to this subject. By far the most detailed account which we possess is that of Strasburger embodied in his two memoirs upon the cell-wall (1) "Ueber den Bau und das Wachsthum der Zellhäute" 1882 pp 95–100. (2) "Ueber das Wachsthum vegetabilischer Zellhäute" 1889 pp. 36–46)

In spite of this attention our knowledge of the development of these anthers is still incomplete & it was the purpose of the present research to re-examine the subject & if possible, to add a few details to the existing accounts. The species which have been examined are Oenothera longiflora, O. biennis & Gaura Lindheimeri. Epilobium tetragonum & E. montanum have also been examined but less thoroughly.

The early development of the anther takes place in quite the usual manner. ~~by the division of~~ A single longitudinal row of hypodermal cells (the archesporium) divide into an inner series of primary sporogenous cells & an outer row of primary parietal cells.(1)

In the latter a succession of periclinal divisions follow one another until, usually, four layers of cells separate the column of primary sporogenous

(1) The terminology used here is that given in Coulter & Chamberlain's "Morphology of Angiosperms" 1903 p. 33.

cells from the epidermis. The outermost parietal layer is the endothecium or fibrous layer; within this follow (usually) two "middle layers" + finally, adjoining the sporogenous tissue, the tapetum becomes differentiated. As is well known the primary sporogenous cells of Gaura form a single longitudinal row. Subsequently certain of these sporogenous cells become "sterile" + by their division, form transverse Septa here + there, ~~along~~ across the anther. The formation of these Septa in certain members of the Onagraceae has already been described by Barciana (Inaug. Diss. Leipzig 1874 "Unters. über die Blüthentwick. d. Onagraceae" p. 21) + by Bower (Studies in the Morphology of Spore-producing Members. II Ophioglossaceae 1896 p. 1)

Large raphide-sacs occur in the connective of all the species examined. The bundle of crystals of each sac is enveloped in a mucilage

which stains violet with a mixture of methylene blue + fuchsin + pink with ruthenium red. These reactions indicate a pectic body. The crystals + their mucilage sheath do not fill the entire sac but the space which is left between them + the wall of the sac is ~~even~~ occupied by a material which has often ~~has~~ a reticulate structure. In Heidenhain's Iron-haematoxylin the mucilage sheath becomes black (Fig 14). whilst the reticular investment remains uncoloured. ~~This more reticulate matrix substance is probably~~ ~~cytoplasmic~~ ~~in nature.~~ No starch or no plastids were ~~even~~ ~~seen~~ ~~even~~ ~~occur in the raphide sacs~~ in the sacs which enclose raphides

Warming in his description of the anther of Epilobium angustifolium (Unters. ueber pollenbildende Phyllome + Kaulome. Bonn 1873. p. 23) calls attention to certain large, ellipsoidal cells which lie in the connective but the nature of which he left undetermined. These cells are the raphide-containing sacs mentioned above which reach quite a remarkable development ~~degree~~ in the species of Epilobium.

In Oenothera biennis & O. longiflora tannin also occurs in the anther, both in the epidermis & in a varying number of cells of the connective. On each side of the anther, along the line of future dehiscence, a longitudinal band of epidermal cells always remains free from tannin. The cells of these two lateral, tannin-free bands soon ceases to grow & become stretched & flattened by the enlargement of the anther. Beneath each of these two lines of peculiar epidermal cells a longitudinal air-passage is formed at a very early stage (1). This passage arises, in the first place, by a separation of cells from one another at these spots but subsequently the cavity is enlarged by the cells bordering upon the space becoming flattened & destroyed by the growth of the anther.

(1) Some time before the appearance of the callose mother-cell walls.

In some anthers a curious development of the cell-walls bordering upon the air-passage was observed. ~~In these cases the~~ The cell-walls in question had become greatly thickened, ~~at some spots enormously so,~~ & cuticularised in a manner which was quite similar to that found in the walls of the pollen grains themselves. The air-passage in these anthers was therefore completely shut off by a continuous mantle of thick, cutinised membranes. The thickening & cuticularisation of these walls had taken place very early in the history of the anther long before the pollen-walls themselves had undergone ~~these~~ such changes & ~~as~~ indeed before the pollen-wall had put in its appearance at all. (Fig. 1)

Stomata occur upon the anther but they are not very abundant. In anthers at about the time when the pollen-mother-cells are established the development of the stomata can be readily followed. It is seen that an initial cell is cut

off from certain of the meristematic superficial cells of the anther & this becomes the direct mother-cell of the stoma (Figs 2, 3 + 4). Starch can nearly always be found in the guard cells of the stoma although the other epidermal cells are quite free from this substance. (1)

I have examined the anthers of Gaura Lindheimeri soon after the primary sporogenous cells have become definitely established by means of the cell-wall reagents recommended by Mangin.

I find that both pectic bodies & cellulose are present in the walls of the anther-cells at this time but that the cellulose is ordinarily masked by the pectic constituent. It is only after treating the sections with dilute acid followed by the action of dilute alkali that the cellulose can be clearly demonstrated. The walls of the sporogenous cells & of the tapetum contain less cellulose than the other regions of the anther.

(1) a little starch occasionally occurs in the epidermal cells of the connective just over the vascular bundle, but never in any other part of the epidermis.

The walls of the primary Sporogenous cells are at first no thicker than those of the surrounding tissues but they soon increase in thickness & stand out conspicuously from the neighbouring membranes. The very young anther contains only a trace of starch in fine granules. The occurrence of starch can first be detected in the filament of the stamen, it then spreads upwards to the cells of the connective which lie dorsal to the vascular bundle & it can next be seen in the primary sporogenous cells. This is the usual sequence of starch appearance but the conditions under which the plant has been grown & the time of day when the anthers have been fixed exercise, at all stages of development, considerable influence over the starch-contents of the anther. Certain broad facts of starch-distribution, however, remain fairly constant in healthy plants grown under average conditions.

In Salvia the single longitudinal series of primary sporogenous cells

~~single longitudinal division of~~

~~In Spaeni the primary sporogenous cells, which have been cut off from the hypodermal cells,~~

become ~~pass~~, without any further longitudinal division, ~~into~~ the single column of pollen-mother-cells, each of which becomes surrounded by a mucilaginous wall of peculiar nature. ~~chemical composition~~. In Epilobium tetragonum the primary Sporogenous cells undergo a single longitudinal division so that two rows of mother-cells are formed whilst in Oenothera a second longitudinal wall often follows the first so that either two or three mother-cells are seen in the transverse section of each pollen sac. The next important step in development is the formation of a mucilaginous ~~wall~~ well round each mother-cell. ~~From its first appearance this wall gives none of the reactions of either cellulose nor of pectose but its behaviour both towards dyes & solvents are those which Mangin has shown to be characteristic of callose.~~

This wall is essentially similar to that which occurs in a corresponding position in other Angiosperms. Mangin[1] & [2] examined the mother-cell walls of a number of flowering plants & concluded, from their microchemical behaviour, that they consist of callose in a peculiarly pure state. In Gentiana officinalis & Campanula rapunculoides Mangin[2] noted some variations in the composition of the (special-) mother-cell wall.

From the facts that this wall, in the Onagraceae, stains deeply with a solution of corallin in soda (4% Na_2CO_3), with aniline blue, benzo-purpurin & congo red, that it gives none of the cellulose reactions with Iodine reagents & is insoluble in cuprammonia, & that it has no affinity for ruthenium red, I agree with Mangin in considering callose to be the only constituent of this wall.

(1) Mangin "Observations sur la membrane du Grain de Pollen mur" Bull. Soc. Bot. d. France T. 36 1889

(2) " "Observations sur le développement du pollen" Bull. Soc. Bot. d. France T 36 . 1889

In several respects, however, I find the reactions of the mother-cell wall to disagree from those usually attributed to callose. Callose is described as readily soluble in 1% caustic potash or soda. I find this statement to require some modification with regard to the mother-cell wall. The (special) mother-cell wall of fresh material of Aucuba japonica dissolved with exemplary rapidity in 1% caustic soda but I have kept microtome sections of material of Oenothera fixed with Flemming's solution for over an hour in 1% caustic potash & still found the mother-cells undissolved at the end of that time. The mother-cell walls of fresh material of O. biennis had only disappeared after nearly 24 hours in 1% NaOH. I have found fresh material of the pollen-mother-cells of the Horse-chestnut equally resistant to 1% caustic alkali. In 10% caustic potash the mother-cell walls of Oenothera soon disappear. It will be seen from these remarks that there is some variation in the solubility of the mother-cell wall in dilute caustic alkalis.

Mangin has affirmed that callose is soluble in phosphoric acid but I have left the mother-cell walls of Oenothera biennis for many hours in strong phosphoric acid without obtaining any signs of their dissolving. Solution.
Naphtol black, in acid solution, is said by Mangin to stain cellulose but to leave callose uncoloured. ~~but~~ I have obtained precisely the opposite result. ~~I have used either an acid or a neutral solution of naphtol black~~

Bismarck brown, methyl ~~green~~ blue & fuchsin are all described as pectic stains which leave callose uncoloured. I have found them all to stain the mother-cell walls ~~but~~ although not ~~so~~ so deeply as the pectic ~~walls~~ membranes.

The origen of callose has formed the subject of repeated discussion. In the case of the callose of the Sieve-tubes some have asserted that this substance arises from the transformation of pre-existing cellulose, whilst others believe it to be a direct product of protoplasmic activity originating without any relation to cellulose or other fore runner. Hill in his account of the Sieve-tubes of Pinus,[1] takes up an intermediate position & believes that callose may originate sometimes directly & ~~sometimes indirectly~~ (?).

In the case of the callose composing the pollen mother-cell walls there can be no doubt concerning its mode of origen.

It has already been mentioned that in the very young anther the walls of the primary Sporogenous cells are poorer in cellulose than the other tissues of the anther. In somewhat older anthers, but still long before the mother-cell

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[1] "The Histology of the Sieve tubes of Pinus" Ann. of Bot. Vol XV 1901 p. 597

Moreover the cellulose which occurs in the young wall
is merely a trace & could not give rise to the massive
callose wall

wall may be expected to appear, the membranes of the sporogenous cells no longer show any traces of cellulose but this, if it still occurs (which is doubtful), is completely masked by the pectic bodies which are strikingly developed in these membranes. Even after treatment with dilute acid & alkali — as recommended by Mangin — I was unable to ~~find~~ demonstrate any cellulose in these walls.

It is within these walls that the callose layer ~~makes its appearance~~ is developed. There is, here, no disappearance of either cellulose (1) or pectose to account for a transformation of these substances into callose. Whatever may be the explanation of the formation of callose in sieve-tubes, I think there can be no doubt that in the case of the pollen-mother-cells the callose is derived directly from the activity of the protoplast without the intermediation of cellulose.

(1) The cellulose is lost sight of in the walls of the sporogenous cells too long before ~~the~~ the callose appears for these substances to have any connection with one another.

Each mother-cell now divides to form the four Special-mother-cells. The mitotic figure is rather small + not well adapted for studying the details of nuclear division. I will content myself, therefore, with stating that in Oenothera longiflora the number of chromosomes which appear at the first & second divisions of the pollen mother-cell is seven. They are so small in size that I can only distinguish them as somewhat irregular granules; whether they have a definite & constant shape peculiar to each division (as seems likely) could not be certainly determined. (Figs 5-9)

In the somatic divisions (which I have studied in the wall-cells of the anther) the chromosomes have the form of curved rods which are crowded together upon the spindle so that it is not difficult to count them. ~~I have distinctly counted easily made out thirteen more than ten + their number will probably prove to be fourteen.~~ I have distinguished 13 to 14 in some cases + the latter number will probably prove to be the correct one.

~~Mother cell wall.~~

Between the cells of the tetrad, which results from this division, Septa are developed which form an extension of the mucilaginous mother-cell wall. Like the latter these Septa also give the reactions of callose. (Figs 10 & 11)

Mangin[1] has called attention to three delicate lines which run through the ~~centre~~ middle of the Septa of the fully grown Special-mother-cell wall & join one another at the centre of the tetrad. ~~in the case of Althaea rosea.~~

~~He pointed out that they~~ These lines were often granular in structure & he believed them to be nitrogenous in nature.

Other authors have figured these radiating lines in the Special-mother-cells of other plants; Strasburger figuring them both for Althaea rosea & Gaura biennis as long ago as 1882.

I have observed these lines in all the

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1) Bull. Soc. Bot. d. France T. 36. 1889. p. 391
Mangin describes this in the Special-mother-cell wall of Althaea rosea.

On agraceae which I have examined. By careful focussing & by the comparison of series of microtome sections I find these lines to be the optical expression of laminae. Most probably these laminae represent the first lamellae deposited after the completion of cell division. ~~They behave somewhat differently towards reagents~~ They differ somewhat from the rest of the special-mother-cell wall in their behaviour towards stains but their reactions still indicate their callose composition (Figs 12+13). Moreover, at a later stage, when the special-mother-cell wall breaks down & the pollen grains are liberated, these lamellae remain behind for some time unchanged & continue to give a very characteristic callose reaction with corallin-soda (Figs 51+52), ~~whilst the rest of the wall which has disintegrated no longer has any affinity for this dye.~~

In anthers which are a little older we observe the first appearance of the pollen membrane round each special-mother-cell.

We first recognise it as a very delicate film lining each cell-cavity of the tetrad. It is in most intimate contact with the callose wall + even reagents which cause general plasmolysis + considerable distortion of the cell-walls of the another seldom separate the very young pollen membrane wall from the special-mother-cell wall. The protoplast of the cell is also firmly attached to the new membrane but it is easier to tear away the cytoplasm from it than it is to separate this film from the callose wall. From the first, however, it can be distinguished from the special-mother-cell wall by its behaviour towards reagents. It stains red with ruthenium red; it colours much more deeply than the callose wall with bismarck brown, fuchsin, or methylene blue; it is unstained by corallin soda, + becomes yellow or brown in chlor-iodine Chlor-zinc-iodine solution.

When these tetrads at this stage are treated

with 10% KOH. the ~~callose~~ callose wall is dissolved + the protoplasts, each surrounded by the undissolved young pollen ~~wall~~ membrane, are set free.
~~the peripheral layer of the tetrad behaves in a similar manner. Although the~~ delicate

We may conclude from these reactions that the young pollen wall is composed of a pectic substance. The remains of the primary sporogenous cell wall, which ~~has~~ is also ~~of a pectic nature~~ gives the reactions of a pectic body, can still be distinguished at the periphery of the tetrad. Although the association between the newly developed pollen membrane + the special-mother-cell wall is so close the demarcation between the two is always sharp + there is never a gradation of one into the other. Where the callose wall abuts upon the pectic membrane it is denser + ~~more highly refractive in appearance~~ refracts the light more strongly than the rest of the wall probably forming a "grenzhäutchen" in Strasburger's sense.

The facts show that although the pollen wall is at first deposited in close contact with, & probably in actual attachment to, the Special-Mother-cell wall it is not derived from a transformation of the innermost lamellae of this callose wall but is directly secreted as a pectic layer by the cell-protoplast. It is equally certain that the plasmoderma(1) is not bodily transformed into the pollen wall but that this is deposited upon the surface of the plasmoderma, as Strasburger has shown in other cases. As soon as the pollen membrane becomes slightly thicker it separates readily from the callose layers & is then clearly recognised as an independent structure. The young pollen grain is a bluntly triangular, basin-shaped structure with the concavity of the "basin" directed towards the centre of the tetrad. It measures about 19 to 20μ across its broadest surface in O. biennis. At the apex of the three angles of the pollen grain the wall is extremely thin. The protoplasm fills the cell-cavity & contains a considerable quantity of Starch. (Figs 15+16)

In pollen grains which are a little older

(1) I use this term as the equivalent of the german "Hautschicht". The word was ~~first~~ suggested for this purpose by Strasburger & first used by Stevens in his paper upon "Gametogenesis & Fertilization in Albugo" Bot. Gazette XXXII 1901 p. 92.

in O. biennis (1)
(measuring about 22 to 24 μ across) the wall
has thickened considerably & a mucilaginous
material has been developed at the three angles
of the cell at those spots which ~~at this period~~
~~stage~~ previously were thin (Fig 17). This mucilage
gives the reactions of a pectic substance &
appears to be derived from the ~~wall~~ growth &
~~growth~~ alteration of the pollen wall at these points.
The little plugs or discs of mucilage continue to
enlarge & soon bulge so far within the cell
that they overlap the unswollen pollen wall
on each side. ~~At first the reactions of~~
~~these mucilaginous areas~~

The name of "Zwischenkörper", introduced by
Fritsche, has been used by Nägeli &
Strasburger in describing these peculiar
mucilaginous discs of the Onagraceous pollen
grain. I shall speak of these discs
as "interstitial bodies" in the present paper.
~~In the large majority~~ There is the normal

(1) In stating the ~~measurement~~ size of the pollen grain I have always taken the measurement across the broad face of the grain from the tip of an interstitial body to the outer surface of the wall immediately opposite.

number of interstitial bodies possessed by the pollen grains of all the species of Onagraceae which have been examined. In a few cases, however, I have noticed four or even five of these bodies whilst in others only two or one interstitial body occured (Figs. 21 & 22) An interesting abnormality has been noticed in some pollen grains of this age. Instead of the single nucleus which is normal at this time pollen grains have been seen which contain two nuclei: a large one & a small one (Fig 18). The case is probably to be compared with the irregularities which Juel(1) & others have described in the nuclear division of the pollen-mother-cells of Hemerocallis fulva & is no doubt due to one or more chromosomes becoming separated from the rest & forming an independent nucleus.

There appears to be some variation in the exact time when the special-mother-cell wall breaks down & sets free the pollen grains.

(1) Juel. O. H "Die Kerntheilungen in den Pollenmutterzellen von Hemerocallis fulva etc" Prings. Jahrb. f. wiss. Bot XXX 1897 p. 205

A large number of my preparations of Oen. biennis show this to occur at the comparatively early age that we are now considering (viz: pollen 22–24 μ across). As was remarked above the first-formed laminae of the special-mother-cell wall maintain their ~~integral~~ individuality the longest & continue to give callose reactions for some time (Figs 51+52). The rest of the wall now forms a homogeneous mucilage filling the pollen-sac & occupying all the spaces between the pollen-grains. It no longer has any affinity for coraliin-soda & its reactions furnish no clue to its chemical nature.

As the pollen grains continue to develope the interstitial bodies become more prominent towards the exterior, giving the broader face of the grain a more pronounced triangular outline.

A secondary thickening layer is now ~~depos~~ formed within the first pollen wall. ~~This~~

This layer extends over the whole inner face of the first membrane of the pollen grain. It runs up the sides of each interstitial body as a cylindrical extension which gradually thins off as it approaches the apex of the body & dies away altogether at the summit itself (See Figs. 19 & 23).

The ~~chemical~~ microchemical reactions of the thickening layer do not exactly correspond with those which are characteristic of any of the ordinary cell-wall components & its chemical nature must for the present be left an open question. With a rather strong solution of Iodine in potassium iodide it gives a very beautiful violet colour but with chlor-zinc-iodine & with a calcium-chloride solution of Iodine it tinges only faintly yellow or yellow-brown. Congo-red leaves it unstained. ~~Pollen grains which have been stained with a red~~ A Methylene-blue & fuchsin mixture stains the layer pink or violet.

I have found that the first pollen-wall of the Horse-chestnut

in its early stages, gives reactions which are almost identical with those of the secondary layer of Oenothera. Apart from the very striking violet reaction with the Iodine solution the properties of these membranes correspond fairly well with those characteristic of pectic substances & it is not improbable that we are here dealing with an association between a pectic body & a substance of unknown nature. Additional support is given to this view by the fact that the violet reaction becomes lost after treatment of the pollen grains with absolute alcohol, no doubt because the body which gives this reaction is soluble in alcohol. In its behaviour towards other reagents, however, the thickening layer remains unaltered after an immersion in alcohol. Cuticularisation takes place very early in these membranes (1) & the violet-reacting body may be associated with the first stages of this process.

(1) after which they colour yellow to brown with Iodine in potassium iodide solution.

In alcohol material the thickening layer, at the early periods of its development, is often greatly swollen[1] + this becomes more marked + may even lead to the bursting of the pollen grains if this is examined in aqueous solutions.

The interstitial body is now[2] ~~(in pollen grains measuring 36 to 38 μ in Oen. biennis & Oen. longiflora)~~ limited towards the cavity of the grain by a closing disc which has the same composition as the rest of that body although it is somewhat tender. The reactions of the whole interstitial body have undergone a change + are no longer those of a pure pectic body. With Iodine-reagents it colours yellow; with congo red it stains uniformly red; with naphtol black it colours blue-black; with nigrosin it becomes black; with ruthenium red it ~~has~~ stains red; with methylene blue-fuchsin mixture it colours blue, pink or violet according to the strength of the solution used; with corallin-

(2) Pollen grains measure at this time 35 to 38 μ in Oenothera biennis + O. longiflora

(1) This was already noticed by Strasburger in Gaura biennis

-Soda solution it remains colourless.
The protoplast fills the cavity of the pollen grain at this stage but weak plasmolysing agents show that, whilst it is firmly fixed to the developing Secondary layer, it is free from the bases of the interstitial bodies.
As the thickening layer of the pollen wall continues its development ring-shaped ridges make their appearance at the bases of the interstitial bodies. These are at first low + inconspicuous but soon become sharp + prominent features on the membrane. (Figs 23 + 24)
In pollen grains of Oenothera longiflora (1) which measure from about 40μ to 45μ the protoplast still completely fills the cell-cavity it but has become entirely free from its walls. ~~The diameter of the pollen grain now rapidly enlarges~~
The further increase in the size of the pollen grain which now takes place is more rapidly than

(1) although I give here the actual description + measurement of the pollen of Oenothera longiflora the facts are essentially the same in O. biennis. ~~the measurements~~

that of the living protoplast which ~~is~~ consequently ~~is~~ no longer fills the cell-cavity (Fig 24). We have here, in fact, conditions which strikingly recall those which Fitting (1-4) & others have described in ~~the case of Isoetes & Selag~~ the case of the megaspores of Isoetes & Selaginella.

These results have such an important bearing upon our conceptions of the growth of vegetable membranes & render some features of this process so difficult to understand that several botanists have hesitated to accept them until they could be placed upon a broader basis than

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(1) Fitting H. "Bau u. Entwickelungsgeschichte der Makrosporen von Isoëtes & Selaginella etc" Bot. Zeit. 1900 Bd 58 pp. 107-164.

(2) Denke P. "Sporenentwickelung bei Selaginella" Beihefte 2. Bot. Centr. Bd XII 1902 p. 182

(3) Lyon M.F. "A Study of the Sporangia & Gametophytes of Selaginella apus & S. Rupestris". Bot. Gazette Vol XXXII August-September 1901 pp 124-141 & pp 170-194

(4) Campbell. H.D. "Studies on the Gametophyte of Selaginella" Annals of Bot. Vol XVI 1902 pp. 419-428.

was done by those who have examined the megaspores of the Lycopodiales.

With the exception of Fitting, these authors have exclusively rested their conclusions upon microtome sections. Invaluable as such sections are we must not overlook the fact that the long series of manipulations necessary for killing, fixing + embedding in ~~paraa~~ paraffin introduce many possible Sources of error + the results obtained by this means ~~must~~ should be carefully checked by observations upon living material.

Fitting worked largely with living Spores which he examined partly in physiological Salt solution + partly in water. ~~The latter is unquestionably an unsuitable medium for this work.~~

Unfortunately he gives us no details of his ~~observations~~ methods + it would be very desirable to know exactly what was the strength of his physiological Salt Solution ~~was~~ + whether this particular concentration was found by direct Experiment

to produce less change in the cell than any other strength. ~~Water was a most unsuitable~~

His selection of water as an alternative medium in which to examine the condition of the protoplast was most unsuitable ~~& no reliance can be~~ ~~be placed~~ as water is ~~well~~ known to affect the protoplasm & its ~~plasmo~~ osmotic condition. ~~in a large number of cases.~~

The pollen grains of Oenothera are particularly favourable for investigation & I have attempted to make my examination of them as complete as possible. Fresh material has been examined in the first place & the results thus obtained have been compared with microtome sections of material fixed with strong + weak Flemming's solutions, with ~~strong~~ & medium chrom-acetic solution (1), Merkel's fluid & Worcester's fluid (2).

The microtome sections were particularly useful

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(1) Formulae in Chamberlain's "Methods in Plant Histology"

(2) Formula for this fluid was obtained from H. S. Reed's paper upon Enzyme secreting cells of Yucca & Phoenix. Ann. Bot. April 1904 p. 271 p. 28

in showing the exact relations which exists between the pollen grains + the other cells of the anther at the different periods of development. I will give here a few of the measurements which I have made of the pollen grain, its cell-cavity + its protoplast. The stamens were examined directly after the removal of the flower-buds from the plants which were all strong healthy ~~plants~~ individuals growing upon an open plot of ground. The pollen grains were carefully teased out of the anther into a drop of the fluid which was being studied + ~~each~~ rapidly examined whilst still uncovered.

I. The stamens from one bud were successively examined in the following solutions (O. longiflora).

(1) .6% NaCl

Pollen grain = 42 μ
pollen cavity = 30 μ
pollen protoplast = 30 μ

(2) .75% NaCl

Pollen grain = 46 μ
" cavity = 30 μ
" protoplast = 30 μ

(3) 2% NaCl

This caused complete plasmolysis

Pollen grain = 40 μ

" cavity = 26 μ

" protoplast = 18 μ

(4) Egg-white

Pollen-grain = 40 μ

" cavity = 26 μ

" protoplast = 26 μ

The results in this reagent were particularly uniform.

(5) Strong Flemming's Solution

(a) Pollen grain = 42 μ

" cavity = 28 μ

" protoplast = 26 μ

(b) Pollen grain = 42 μ

" cavity = 28 μ

" protoplast = 28 μ

(6) Medium chrom-acetic

Caused slightly more shrinkage than the Flemming's sol. Pollen grain = 44 μ

" cavity = 30 μ

" protoplast = 26 μ

(6) Strong chrom acetic solution

Gave results similar to the Flemming's solution

(7) (8) Merkel's solution

Pollen grain = 40 μ

" cavity = 30 μ

" protoplast = 30 μ

Although difficult to recognise at this stage Merkel's sol caused the protoplast to swell up & enlarge somewhat.

II The stamens from another bud were examined in

(1) .75% NaCl

Pollen grain = 62 μ . ~~66 μ~~ . 68 μ

" cavity = 36 μ . ~~36 μ~~ . 36 μ

" protoplast = 24 μ . ~~30 μ~~ . 30 μ

(2) Egg-white

Pollen grain = 62 μ . ~~70 μ~~ . 70 μ

" cavity = 36 μ . ~~42 μ~~ . 36 μ

" protoplast = 26 μ . ~~38 μ~~ . 32 μ

(3) Strong Flemming's Solution

Pollen grain = 64 μ . 70 μ
" cavity = 38 μ . 40 μ
" protoplast = 26 μ . 30 μ

(4) Merkel's Solution

Pollen grain = 66 μ
" cavity = 40 μ
" protoplast = 40 μ

This caused the protoplast to swell up.

(5) Strong Chrom-acetic solution

Pollen grain = 66 μ
" cavity = 36 μ
" protoplast = 28 μ

III Stamens from another bud examined in
(1) .75% Nacl

Pollen grain = 80 μ
" cavity = 46 μ
" protoplast = 32 μ

(2) Examined in a drop of juice squeezed from the stem of Datura longiflora.

Pollen grain = 76 μ . 76 μ . 80 μ
" cavity = 42 μ . 44 μ . 46 μ
" protoplast = 32 μ . 36 μ . 34 μ

(3) In 5% cane-Sugar Solution

Pollen grain = 72 μ
" cavity = 42 μ
" protoplast = 30 μ.

IV In another bud ~~stamens~~ pollen examined in a drop squeezed from stem of Datura. longiflora:

Pollen grain = 72 μ
" cavity = 42 μ
" protoplast = 34 μ

V. In another bud pollen was examined in:-

(1) ·6% NaCl

Pollen grain = 62 μ
" cavity = 34 μ
" protoplast = 28 μ

(2) Absolute Alcohol

Pollen grain = 62 μ

~~pollen~~ cavity = not measured

" protoplast = 16 μ

Alcohol always caused great shrinkage of the protoplast. ~~Occasionally often~~ distortion of the ~~pollen membrane~~.

VI The following observations were made upon the pollen grains in the following media:-

(1) The Stamen was placed in a drop of olive oil & the pollen carefully teased out without coming into contact with the air. Pollen grain = 68 μ

" cavity = 41 μ

" protoplast = 28 μ

(2) Pollen grains teased rapidly into distilled water & immediately examined showed a protoplast of 28 μ in pollen grains 68 μ across; Very soon however the vacuoles of the protoplast enlarged, ruptured the separating arms & laminae of cytoplasm & ran together ~~until~~ so

that the ~~whole~~ protoplast slowly swelled up until it quite filled the pollen-cavity (42μ).

(3) In 2% cane sugar the results were similar to those in distilled water.

(4) In 5% cane-sugar

The protoplast for some time maintained its size of 28 to 30μ. Later, however, in some of the pollen grains changes similar to those of (2) & (3) were seen but much less marked.

(5) In .6% NaCl

The protoplast measured about 28μ + it remained unaltered after a prolonged examination

(6) In 2% NaCl

Protoplast measured ~~22μ~~ ~~later~~ only 20μ + was obviously plasmolysed.

The following observations upon O. biennis may also be mentioned.

I carefully & as rapidly as possible teased out the pollen grains into a little of the mucilaginous fluid which can be squeezed from the anther itself. In a pollen grain measuring 68 μ & a cavity of about 40 μ the ~~pollen grain~~ protoplast measured 30 μ. I then added a drop of .6% NaCl solution to the above, ~~& again measured the pollen grain & the protoplast watching~~ watching the effect all the time. The pollen grains both remained unchanged in their appearance & in their measurements.

In another similar experiment upon another bud from a different plant the measurements both in the anther-juice & in the .6% NaCl solution were:—

Pollen grain = 74 μ
" cavity = 40 μ
" protoplast = 30 μ.

I might occupy many pages in quoting similar measurements but as those which I have already mentioned are quite typical of the rest it would serve no useful purpose to do so. The general result has been to show that in .6% NaCl, .75% NaCl, 6% cane-Sugar, & egg-white + the plants own juice the protoplast has a very similar appearance + its measurements agree very well with one another at the different stages Moreover, after remaining in these solutions for some time little or no alteration either in size or appearance, was observable. The average measurement in these solutions calculated from all my notes are as follows:—

Pollen grain	pollen cavity	protoplast
40 μ	26 μ	26 μ
62 μ	37 μ	27 μ
70 μ	39 μ	32 μ
74 μ	42 μ	34.25 μ
80 μ	46 μ	34.5 μ

Strong Flemming's Solution & Strong chrom-acetic solution do not alter the protoplast very much in appearance but usually cause some shrinkage. Merkel's fluid was not so satisfactory & it causes the vacuoles to swell up & the protoplast to enlarge.

Absolute alcohol causes very considerable contraction of the protoplast

Distilled water enlarges the vacuoles & causes them to run together by breaking down the separating arms & laminae of the cytoplasm. Consequently the whole protoplast swells up greatly.

Objection may still be taken to ~~the results obtained~~ conclusions drawn from a study of the living pollen in the plants own juice, the salt solutions & in egg-albumen, on account of the possible ~~effect~~ influence which ~~the mechanical operation~~ the mechanical operation of teasing out the pollen grains may have exerted.

It may still be urged against these observations that the mechanical operation of opening the anthers & teasing out the pollen grains may have caused a plasmolysis of this protoplast. That mechanical disturbances can affect the living contents of these cells is shown by the fact that if the pollen grains, in e.g. .6% NaCl solution, are covered by a cover glass & the pressure due to this is not relieved by the protoplast gradually enlarges & may finally fill the cell-cavity. If the precaution be taken, however, of preventing the pressure of the cover glass by a fragment of anther or filter paper or not covering the preparation at all. No such change takes place in the protoplast. The best answer to this objection is given by the examination of microtome sections from anthers which have been fixed without subjecting the pollen grains to any such disturbance. Of all the fixatives used the Stronger Flemming's solution showed the best preservation of the pollen

An error from this cause, however, is extremely improbable as the pollen grains can be drawn out from the anther without actually subjecting them to the touch of an instrument + with only very little pressure or friction. This can be done by means of the fibrous mucilage which surrounds ~~them~~ the pollen grains & binds them together in long strings.

Moreover microtome sections of pollen grains, ~~still in~~ fixed whilst lying ~~in~~ untouched within the anther, show a rough parallel in their measurements with those described for fresh material. I will not however, ~~Cannot~~ lay ~~much~~ great weight ~~however~~, on the evidence of the microtome sections as, in spite of every precaution, I never succeeded in entirely avoiding shrinkage[1] of the pollen-protoplast even when all the other cells of the anther were uncontracted. I will add here ~~a few~~

~~measurements of several pollen grains from microtome sections.~~

~~Comparison between the measurements of the pollen from of a living anther with those of microtome~~

~~Sections.~~

(1) ~~The Flemming's solution used for fixing the anthers is~~ ~~and no doubt~~ a part ~~responsible for some~~ ~~of this contraction.~~

a comparison between the measurements of the pollen from a living anther with those of microtome sections :—

(1) Fresh material of Oenothera biennis examined in the juice squeezed from the anther :—

Pollen grain = 74 μ
" cavity = 40 μ
" protoplast = 30 μ

(2) Sections of anther ~~fixed~~ of about same age fixed in Flemming's solution :—

Pollen grain = 72 μ · 70 μ · 70 μ · 70 μ
" cavity = 42 μ · 40 μ · 40 μ · 40 μ
" protoplast = 26 μ · 28 μ · 24 μ · 22 μ

During the whole time that the protoplast is separated from the membrane in this way the latter continues to grow both in extent & in thickness. We are at present quite in the dark regarding the manner in which this growth takes place but a very brief theoretical consideration of the subject will be found among the conclusions at the end of this paper. We must now enquire whence is derived the material necessary for this growth.

There are two sources from which the plastic material of the membrane might be derived, viz. the protoplast of the pollen grain itself or the tapetum.

That metabolic processes of no mean order are taking place in the former is evident from a study of the changes which can be observed in it during this period.
Starch appears & disappears in the pollen grain in a manner which shows that carbohydrates are being used up in the cell; the cytoplasm continually grows less & less in amount whilst a liquid, apparently the direct consequence of the foregoing processes, gradually forms in the protoplasm. This liquid first occupies small vacuoles in the cytoplasm, these continue to increase in size & run together (Fig 24) until we find nothing left of the protoplasm but a hollow shell consisting of a plasmoderma (Hautschicht) + a nucleus surrounded by a trace of granular cytoplasm (see Fig 25 which gives a rather later stage). In the centre of the shell is occupied by one enormous vacuole. (1)

There is no reason to doubt that this liquid

(1) Strasburger in his work of 1882 already wrote of Gaura biennis " in meinen Alcoholpräparaten bildet der nach Anlage der Wand erschöpfte Inhalt der Pollenzelle nur noch ein unscheinbares Klümpchen" cp. his Figs 48 & 49 Tafel VI

diffuses out from the protoplast into the space which has formed is forming between itself + the pollen-wall +, in all probability the latter derives the necessary materials for its growth from this source.

T16 Unfortunately I could gain no knowledge whatsoever of the chemical nature of this liquid.

In the tapetum we can also observe evidences of metabolic activity but I can find nothing to show that any of the material which is being formed there is leaving the cells, on the contrary there is reason to believe that an accumulation of substance is taking place.

In the very young pollen grain (1) the first wall appears as a single homogeneous lamella but when the grain has grown + measures about 40 μ across we can indistinctly recognise a structural differenentiation in the outer membrane

(1) This description of the pollen wall applies both to Oenoth. longiflora + to Oen. biennis unless specially stated to the contrary. The measurements more particularly refer to O. biennis but the dimensions are only very slightly different in the O. longiflora.

When the diameter of the pollen grain has increased still further (to about 55 to 60 μ) its first membrane can be clearly seen to consist of a thin, outer, homogeneous layer + an inner "rodlet" layer (Stäbchenschicht or anschlusslamelle)[1]. The growth of this membrane recalls Strasburger's[2] description of the first pollen wall of Althaea rosea which, at a certain stage, was seen to consist of three lamellae: a middle "rodlet" layer (anschlusslamella) which is bounded peripherally by two homogeneous layers. Of these the innermost lamella soon ceases to grow + becomes gradually more attenuated until it is ~~quite~~ lost sight of altogether; the two other lamellae increase in thickness + the "rodlets" can be very clearly studied in older stages. A thickening layer is developed within the first pollen membrane of Althaea.

In Oenothera the first wall is so thin during

(1) Strasburger "Die pflanzlichen Zellhäute" Prings. Jahrb. f. wiss. Bot. Bd XXXI 1898 p 551

(2) l. c. p. 555

its early development that I have not been able to determine whether the "rodlet" layer is ever bounded internally by an inner homogeneous lamella; it is certain, however, that by the time the pollen grain has reached 55 to 60μ in diameter every trace of it has vanished.

The first pollen wall now grows more rapidly in surface than the secondary thickening layer beneath it + consequently it becomes separated from that layer & all parts of only remains firmly fixed to the interstitial bodies. The continuation of this unequal growth in surface gradually throws the outer wall into irregular & sinuous folds. Both primary & secondary layers of the wall have meanwhile undergone a change in their chemical constitution & have become more or less completely cuticularized. The secondary layer no longer gives a pure

Violet ~~reaction~~ colour with a solution of Iodine in potassium iodide but this has changed first to a violet-brown + then to a pure brown reaction. 16 S. 17

The interstitial bodies are shut off from the cavity of the pollen grain by a well developed closing-disc which consists of two parts; an outer dense + homogeneous layer + an inner less dense, stratified lamina which is cup-shaped in form with the concavity directed towards the cell cavity. Lying on the outer lamina of the closing-disc is an aggregation of granular material which extends some little way up the sides of the interstitial body. The cavity of the interstitial body is no longer occupied by the mucilaginous ~~substance~~ deeply staining substance which filled it ~~at an earlier~~ at an earlier period, but it now contains a watery fluid which does not readily stain. I believe that the mucilaginous material of the young interstitial body has been, to a great extent, used

up in forming the closing-disc & that the granular ~~m~~ substance which lies upon the outer portion of the disc is a remnant of the staineable material. (See Figs 23 ~~& 24~~)

In pollen grains which measure between 85 μ & 95 μ in both O. biennis & O. longiflora across the protoplast has become reduced to a hollow sphere or vesicle which ~~also~~ has expanded again until it is nearly or quite in contact with the cell-wall (Fig 25). At one point upon the ~~protoplasmic vesicle~~ is flattened, rather dense nucleus can be seen which encloses a ~~rather large~~ nucleolus. A little finely ~~finely~~ granular cytoplasm surrounds the nucleus but in its other parts the ~~sphere~~ protoplast appears to be reduced to a plasmoderma (Hautschicht) which surrounds the enormous central vacuole. ~~A little very finely granular shell~~ Very soon the nucleus enlarges, & becomes rounder & less dense & passes into the prophases of mitotic division (Fig 26).

I have not followed the details of this division which leads to the formation of two distinct cells within the pollen grain: the large vegetative cell & the small generative cell. The latter is limited by a well marked plasmoderma (Hautschicht) (Fig 24). The tapetum now breaks down & its contents clearly furnish the material for the renewed growth of the pollen-protoplasts.

In order to understand the nature of this material it is necessary to consider the changes which take place in the tapetum during its earlier development.

In the very young anthers, before the full number of primary Sporogenous cells is established, the tapetal cells contain a not very dense cytoplasm which encloses a single nucleus. This nucleus, besides small, scattered chromatin granules, contains one to four nucleoli. The nuclear membrane colours deeply with iron-haematoxylin or with methylene blue-fuchsin

mixture. Very rapidly the cytoplasm increases in density & the originally single nucleus divides into several, as many as eight nuclei being not uncommonly met with in a cell (Figs 30 & 31) Until about the pollen-mother-cell stage the tapetal nuclei multiply by exclusively by mitotic division but at the mother-cell stage nuclear figures occur which are strongly suggestive of fragmentation. When the special-mother-cells have been established mitotic divisions are rarely met with while fragmenting nuclei occur on every side. Most of the tapetal nuclei now contain a single large nucleolus & a very deeply staining nuclear wall, besides this only a very little finely granular chromatic material can be seen lying near or upon the nuclear wall. (See Fig 44)

In anthers in which the first pollen-wall is just making its appearance I have several

times seen the tapetal nuclei in the prophases of mitosis but I have never, at this period, succeeded in finding the later stages of division & I believe that mitosis is no longer completed by the nuclei. Nuclei which have every appearance of undergoing fragmentation are, however, very abundant both at this & at later stages of development (Figs 32, 33, 34, 40, 42) Strasburger (1) & later writers, in describing the tapetum of other plants, have found mitosis to be the only mode of nuclear division & they believe the constricted nuclei also occur in the cells to represent fusion & not fragmentation of the nuclei.

In Oenothera it is impossible to imagine that Karyokinesis can be the only mode of nuclear multiplication.

~~It is incredible that the mitotic divisions which are now very abundant frequent & are entirely limited to the stages of development~~

(1) Strasburger E. "Theilungsvorgang d. Zellkerne etc" Arch. f. Mikro. Anat. Bd 21. 1882 pp. 574-575.

In the first place mitotic divisions are never very frequent & it is difficult to account for the presence of six or seven nuclei in a young tapetal cell through their agency alone.

Moreover, mitotic figures cannot be found in the tapetum of Oenothera after the appearance of the first pollen wall so that if this is the only mode of division & the constricted nuclei which are common both at this & at subsequent stages, really represent fusions it is impossible to see where the constant supply of nuclei comes for these repeated fusions & which leaves the older tapetal cell with two or three nuclei to the last. The way in which these constricted nuclei often hang together by a narrow neck also favours the view that they are separating from one another & not uniting. (see especially Fig 34)

The great disparity ~~in size of the nuclei of a cell, as which can often be noticed in the size of the nuclei~~ which often exists in the sizes of the nuclei of a cell

is also what one would expect with direct (two nuclei in Fig 34 compare sizes of these) rather than with indirect division

For all of these reasons I consider that most of these constricted nuclei represent a fragmentation & not a fusion of nuclei

Every constricted nucleus does not, however, necessarily imply nuclear multiplication. There is no doubt that the tapetal nuclei alter their shape & often become very irregular in outline without this leading to a division of the nucleus or representing a fusion (see Figs 37. 36). These changes in shape are evidently signs of the occurrence of an active metabolism in the cell & may be compared to the similar phenomena which have been described in the secreting cells of many animals.

This continuous nuclear multiplication, by both direct & indirect division, must lead to the accumulation of a large number of nuclei

in each cell unless an opposite process, reducing their number takes place at the same time. A glance at a section of an older anther will at once show that no excessive accumulation of nuclei occurs in the cell + I have ~~not~~ succeeded in finding clear evidence of a nuclear degeneration taking place side by side with the nuclear multiplication. In this process the nuclear membrane, which stains very deeply, becomes ruptured + [shredded out into a groups of fibres or] narrow lamina whilst the nucleolus can also in many instances, be seen to resolve itself into a coarse fibre.

There can be little doubt that the groups of fibres formed in this manner correspond to the structures which Meves[1] has recently described in the tapetal cells of Nymphaea alba & which he has compared to the chondriositen of certain animal cells.

(1) Meves Fr. "Ueber das Vorkommen von Mitochondrien bezw. chondriositen in Pflanzenzellen" Bericht. d. Deutsch. Bot. Gesell. XXII 1904 pp. 284-286

It is quite easy, in well fixed material[1], to find all stages between a complete nucleus & one that is only represented by a group of fibres. In Figs 43, 44 & 45 d & f. I have drawn nuclei which are degenerating in this way.

These fibres, of nuclear origin, become gradually more numerous in older anthers as the tapetal nuclei continue to divide & to degenerate, but whether they all persist as fibres or whether some of them are lost sight of in the course of further changes I am unable to say. It is certain, however, that the cytoplasm of the tapetal cells which are approaching disintegration stains very deeply & that it contains a large number of these fibres.

Just before tapetal disintegration the whole contents of the cell, apart from the unaltered nuclei[2], break down into coarse granules which stain intensely with iron-haematoxylin &

(2) which are two or three in number.

(1) Worcester's fluid is by far the best fixative for this purpose.

these become distributed among the pollen grains when the cell loses its individuality. ~~Many of these granules, especially after they lie free among the pollen grains, appear to undergo a fatty degeneration as they then turn intensely black with osmic acid, either over their entire surface or more often they enclose particles (or drops) which do so.~~

During the development of the anther starch appears & again disappears in the tapetum according to the conditions of growth & this shows that carbohydrates are being employed in metabolism.

The conclusion which may be drawn from the above facts is that a large part of the material which accumulates in the tapetal cells during their development & which subsequently passes into the pollen grains to replenish their exhausted protoplasts has

at one time or another entered into the composition of the tapetal nuclei & that there is here, therefore, a direct relation between nuclear substance & cytoplasmic growth.

The comparison which Meves has drawn between these deeply staining fibres of the tapetal cytoplasm & the chondriositen of certain animal cells is of the highest ~~importance~~ interest.

In a large number of ~~the most~~ very actively functional cells, belonging to the most various tissues of the animal body, chromatic structures have been found in the cytoplasm & described under the names of mitochondrien, chondriositen, pseudochromosomes, yolk-nuclei, chromidien, apparato reticolare etc.

Goldschmidt[1] has recently found good grounds for grouping all these structures together & he has shown by direct experiment that at least in some cases (e.g. muscle-cells of Ascaris lumbricoides) they are directly connected with

(1) Goldschmidt R. "Der chromidialapparat lebhaft functionirender Gewebszellen" Zoolog. Jahrb. Abth. f. Anat. & ontogenie d. Thiere Bd XXI 1904 pp. 1–100.

the functional activity of the cell.
In the tapetum the fibres (or their derivatives) unquestionably play a prominent part in the cytoplasmic growth of the pollen-protoplasts & no doubt in the animal cell they are also in some way associated with the elaboration of complex organic substances(1)? In this relation it may be recalled that ~~certain~~ several physiological chemists have pointed out the probability of nuclein or one of its constituent ~~molecular groups forming a centre or starting~~ point for the synthesis of complex organic matters in the living cell.
The origin & chemical nature of these chromidial structures has, however, not yet been satisfactorily determined in all cases. In some cells which have been studied by Goldschmidt it is highly probable that they are derived from the ~~nucleus~~ chromatin of the nucleus.

(1) For example ~~notice~~ note the relation which Mathews found to exist between the deeply staining fibres & the zymogen granules of certain pancreas & liver cells. Journ. Morphol. XV Suppl. 1899.

I have shown above that the fibres in the tapetal cells of Oenothera possess a nuclear origen & may be referred to the transformation of the nucleoli & nuclear membranes.
The staining reactions & the behaviour of these nucleoli, whilst the nucleus is still intact, show that they are, at least partly, composed of chromatin whilst the nuclear wall also seems to owe its affinity for nuclear dyes to the deposition of finely granular chromatin upon its ~~inner face or within~~ its substance.
We see therefore that the fibres lying in the tapetal cytoplasm are to a great extent derived from the chromatin of the nucleus & that much of the substance that ultimately passes into the pollen grains is a derivative of chromatin.

~~made~~.

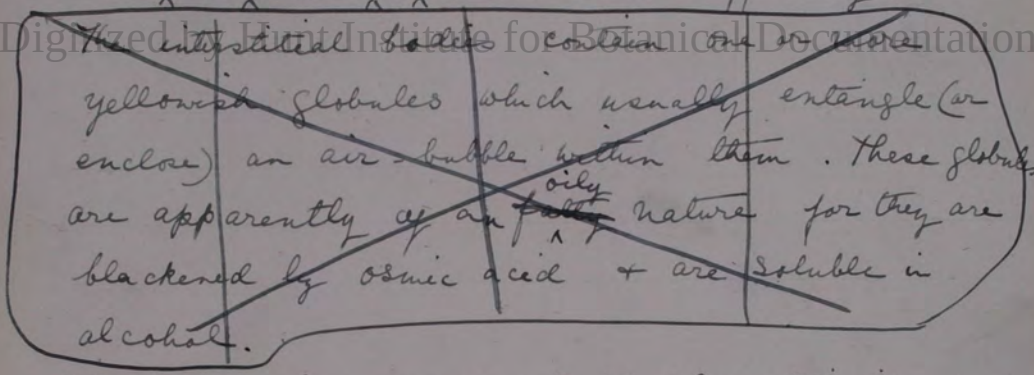
The walls of the tapetum, during the greater part of the development of the anther are of a somewhat mucilaginous nature & can be very distinctly differentiated by means of an alkaline solution of congo red. In the older anther these walls become very thin & at the time when the tapetum disintegrates they become so attenuated that at some ~~certain~~ spots they are apparently interrupted altogether. It is ~~appa~~ obvious therefore that the tapetal walls ~~will~~ offer no great ~~hindrance~~ hindrance to the passage of the cell-contents. It is more difficult, however, to understand how the tapetal substance passes through the thick, cuticularised pollen-wall to reach the protoplast. It must evidently do so in a state of solution but how the complex material~~x~~ of the tapetal cells is brought into solution can at present only be guessed at. Enzymes ~~will~~ are probably ~~be found to~~ be the effective agents but we at present have

no knowledge either of their source or nature. ~~of these substances~~.

We left the pollen grain at a stage when the protoplast, in the form of a hollow shell, had ~~again~~ enlarged sufficiently to fill the cell-cavity once again. At a slightly later period the generative cell + the vegetative nucleus leave their peripheral position for one in the centre of the cell cavity where they are suspended, & together with more or less cytoplasm, by three thick ~~strands of cyt~~ cytoplasm + often several smaller ones as well. The three thick arms of protoplasm extend to the bases of the three intersilital bodies + it is a significant fact that the intine can first be recognised at these spots + that it here attains its greatest thickness. (Figs 28 + 29). It is difficult to avoid the conclusion that influences of some kind originate in the nucleus + pass ~~to the~~ along the three arms of cytoplasm to these spots ~~where the plasmodema is depositing new cell wall lamellae are forming but in~~

~~the peripheral protoplasm~~ at which new cell-wall cavities are forming but we are at present quite in the dark as to the nature of these influences.

In still older pollen-grains, measuring from 108 to 112 μ in diameter, the intine ~~has completed its development~~ forms a continuous layer over the whole inner face of the ~~exine~~ wall: ~~secondary thickening layer of the exine~~. It is thick & easily seen at the base of each interstitial body but it is extremely delicate elsewhere & can only be traced as a continuous membrane ~~demonstrated~~ with some difficulty.



The interstitial bodies contain one or more yellowish globules which usually entangle (or enclose) an air-bubble within them. These globules are apparently of an oily nature for they are blackened by osmic acid & are soluble in alcohol.

The intine gives very clearly the characteristic reactions of a pectic substance but I was ~~am~~ not able to demonstrate the presence of cellulose in it with any certainty. ~~With the Iodine reagents (Calcium chloride Iodine or chlor-zn-Iodine)~~

The interstitial bodies contain one or more yellowish globules which usually entangle an air-bubble in them. These globules appear to be of an oily nature for they are blackened by osmic acid & they are soluble in absolute alcohol.

The protoplasm covered by the intine now bores its way through the closing disc & enters the interstitial body which it soon entirely fills. ~~completely~~. I have followed this process most completely in the case of Gaura Lindheimeri & I will therefore refer to this plant in the present description.

In the quite young pollen grain of Gaura the interstitial bodies are composed of a homogeneous mucilage which in every way resembles that of Oenothera at a corresponding age.

In older grains this structureless mucilage becomes distinctly laminated. These laminae are ~~closest~~ very closely arranged at the base of the interstitial & form there a closing disc[1].

(1) So closely are the laminae arranged in the closing disc that the laminated appearance is often lost sight of altogether & the disc appears granular.

Above the closing disc the laminae are much more loosely placed & they often become drawn out & even broken at their middle by the growth ~~expansion~~ of the interstitial body. At the apex of the interstitial body the laminae again are very densely arranged.

The intine forms quite a thick pad under each interstitial body but is very thin over the rest of the pollen grain. It ~~gives~~ contains both cellulose & a pectic body in its composition. Both substances are distributed equally through the thickness of the membrane & there is no differentiation of a pectic layer from a cellulose one.

When the intine & protoplast are about to penetrate the interstitial body we first find that a narrow cleft is bored through the middle of the closing disc (Fig 46). Then a ~~reason~~ small fold of intine can be seen to pass into this slit (Fig 47) & gradually make its way to the centre of the interstitial body where the laminae are thin

or quite broken through. Here it bulges out into a small, thick-walled sac (Figs 48+49). The laminae of the interstitial body are gradually eaten away & the intine-sac continues to grow until it lies closely against the short teeth which alone remain of the interstitial laminae.

It is interesting to note that the intine must be of a very soft & even mucilaginous nature as it often moulds itself to all the irregularities on the wall & "flows" between the teeth which project from the interstitial wall. The opening in the closing disc gradually enlarges until the disc is reduced to a narrow & dense collar or ring. (Fig 50)

The manner in which the closing disc is perforated & the substance of the interstitial body slowly eaten away suggests the presence of a solvent, probably an enzyme, which is secreted by the protoplast & which carries out the work of disintegration. It is difficult otherwise to

explain the appearance of a clean cut aperture in the closing disc before the intine ~~itself~~ grows out to force itself a way. Moreover, the slow dissolution of the interstitial laminae takes place before the intine comes into contact with them so that they cannot be mechanically broken down by the growth of that membrane.

The mature pollen grain of Oenothera longiflora measures between 170 & 180μ across; it is ~~crowded with starch grains~~ quite filled by the protoplast which is densely crowded with starch. The two layers of the ~~wall~~ exine are again in contact with one another. ~~The "rodlets" or teeth of the outer rodlet layer are not very conspicuous,~~ The outer layer is, however, only firmly attached over the interstitial bodies; it consists of ~~a~~ an outer, ~~and~~ homogeneous lamella which is continuous ~~from~~ over the whole pollen grain & the inner "rodlet" layer which is interrupted over the apices of the interstitial bodies.

During the later growth of the pollen grain the secondary thickening layer has not increased in thickness but has, on the contrary, become stretched & ~~a good deal~~ very much thinner than it was at an earlier stage. (In Fig 50 the ~~exine~~ secondary thickening layer has been drawn too thick)

In Oenothera longiflora all the pollen grains do not reach maturity but a large proportion of them become arrested in their development. They ~~mostly~~ all grow to about 90μ ~~across~~ in diameter, when their protoplast has become reduced to a hollow shell, but ~~then~~ after that many of them are unable to continue ~~their~~ their development owing, no doubt, to the tapetal substance being insufficient for the requirements of ~~&~~ all the pollen grains.

I have not given any special attention to the germination of the pollen grain but I may mention that the intine of Epilobium tetragonum

which gives the reactions of both cellulose & pectose, grows out into a tube which is often branched at its free end (Fig 20)

The mature pollen grains of Oenothera are bound together in long strings by bundles of "fibrils" which lie between & round them. These fibrils are developed from the mucilage which, on an earlier page, we saw was derived from the disintegration of the special-mother-cell walls. These "fibrils" have entirely lost all affinity for callose dyes & have become very resistant to solvents. Their properties in many respects resemble those of cuticularised structures.

In the species of Epilobium short bands of the cuticularised mucilage binds together the pollen grains ~~in tetrads~~ which ~~are liberated in tetrads~~ consequently leave the anther consequently in tetrads.

Summary + conclusions

(1) In the earliest stages of anther development all the cell-membranes contain both cellulose + pectose. The walls of the sporogenous cells, however, contain less cellulose than the other membranes of the anther. In older anthers the sporogenous cell-membranes give the reactions of a pectic substance alone.

(2) The pollen-mother-cell wall consists of pure callose. This substance is formed directly as such by the protoplast + there can be no possibility, in the present case, of a transformation of cellulose into callose.

(3) In the first + second divisions of the pollen-mother-cell seven chromosomes occur, whilst in the somatic divisions fourteen is the approximate number of chromosomes. The presence of two nuclei, one large + the other very small, in some quite young pollen grains suggests the occurrence of irregularities in the divisions similar to those described by Juel in Hemerocallis fulva.

(4) The first pollen membrane is formed by the direct activity of the protoplast + is deposited as a delicate layer of pectic material upon the inner face of the special-mother-cell wall. Although it originates in the

most intimate contact with the callose wall it is chemically distinct from this from the very first.

(5) The interstitial bodies originate as specialised ~~spots~~ areas on the first pollen wall. These spots are at first characterised by their greater thinness; later a homogeneous mucilage is developed at these ~~points~~ places. In ~~old~~ older pollen grains a portion of this mucilage is deposited as a dense closing disc whilst, in Oenothera, the rest of the interstitial body is filled with a thin fluid. In Lavatera more or less solid laminae are deposited throughout the interstitial body.

(6) A Secondary thickening layer is laid down by the protoplast within the first pollen membrane This layer gives most of the pectic reactions but also a very distinct violet colour with a strong solution of Iodine in potassium Iodide. It gives none of the usual cellulose reactions. ~~However~~.

(7) Both the first pollen wall & the Secondary thickening layer are firmly attached to the

protoplast when they are first developed.
In pollen grains which have reached 40μ in size the protoplast is no longer fixed to the wall at any place although it still completely fills the cell cavity.
The pollen grain continues to grow & its walls increase both in thickness & in extent.
Whilst the pollen grain doubles its diameter the cell-cavity increases in size from about 26μ to about 46μ. The protoplast, however, grows far less rapidly during this time & its diameter only enlarges from 26μ to about 34μ.
During the period of the greatest growth of the cell-wall, both in thickness & in extent, the protoplast is, therefore, completely separated from it.
Either the cell-wall can grow by intussusception without the direct influence of the living element of the cell or that element can exert its influence across a space filled with fluid.
The latter assumption is, however, very unlikely.

In consequence of this inequality in growth the protoplast becomes separated from the pollen wall by a space which is filled with liquid.
The conditions seem to be quite similar to those which Fitting & others have described in the megaspores of Isoetes & ~~Lab~~ Selaginella.
In Oenothera also, as in the megaspores, the growth of the layers of the wall is not equally rapid & the first pollen wall becomes separated from the secondary thickening layer & is thrown into folds upon its surface.

These observations show that during the period of most active growth of the membrane (both in surface + in thickness) the protoplast is completely separated from it + we must conclude either that the growth of a cell-wall is a purely physical process or that the living protoplast can exert its influence across a space filled with liquid.

I may add here that although the growth of a membrane whilst this is separated from the protoplast by an actual space has only been found to occur in the few isolated cases mentioned above other less extreme instances belonging to the same category of phenomena are not unknown. Wherever we find that a new lamella is interpolated between the protoplast + an older lamella + the latter still continues to grow in thickness or in surface it does so whilst it is neither in union or in contact with the living element of the cell.

Meanwhile changes are taking place in the protoplast & we find that a fluid is forming ~~there~~ in the cytoplasm, partly at the expense of carbohydrates which reach it from without & partly at the expense of the cytoplasm itself. This fluid is formed in vacuoles which gradually run together until the protoplast is reduced to a hollow sphere enclosing a single, large, central vacuole. We have reason to believe that this fluid diffuses out from the protoplast & furnishes material for the growth of the pollen-membrane.

The three most important features in the formation & development of the layers of the pollen wall may, therefore, be summarised as follows: —

(i) Both the primary pollen wall & the secondary thickening layer originate in intimate connection with the plasmoderma (Hautschidt).

(ii) The greater portion of the growth of both these membranes takes place by intussusception whilst they are completely separated from the protoplast.

(iii) The material required for the growth of the membranes is derived from the secretory activity of the pollen-protoplast.

We can at present only vaguely guess at the most probable way in which the growth of these membranes takes place.

There are some facts, such as Ambronn's work[1] upon ~~Cotoneaster~~ the optical properties of the cuticularised walls, which indicate that the cell-wall may be ~~under laid by a crystalline structure~~ & it is possible that when the membrane is first formed the protoplast (to which ~~the~~ it is then firmly fixed) determines the character & the arrangement of these crystals.

~~So long as the material necessary for the growth reaches it each member of this crystalline system continues to add new molecules to its substance just as in the growth of any other crystal.~~

~~A crystal of alum does when it is suspended in a solution of that substance.~~

(1) Ambronn H. Ber. d. Deutsch. bot. Gesell. 1888 p. 226.

The later growth of the membrane, even after it has become separated from the living element of the cell, takes place in a manner which ~~in determined~~ depends upon the nature & relative positions of its crystalline components.

(8) After the pollen-protoplast has become almost completely exhausted by its secretory activity its substance is once more replenished by material derived from the disintegration of the tapetum.

The very young tapetum contains a rather scanty cytoplasm & only a single nucleus. At the time when the pollen-mother-cells have become established the tapetal cells are furnished ~~by~~ with a denser cytoplasm & their nuclei are dividing both by mitosis & by amitosis. In anthers which are ~~a little~~ older the tapetal nuclei divide ~~less frequently~~ & exclusively by amitosis.

(8) After the pollen protoplast has become almost completely exhausted by its secretory activity its substance is once more replenished by the material derived from the disintegration of the tapetum.

The very young tapetum contains a rather scanty cytoplasm + only a single nucleus. Later the tapetal cells are furnished with a denser cytoplasm & ~~round~~ nuclei which may vary in number from one to eight. Until the end of the special-mother-cell stage both mitotic & amitotic divisions of the nuclei take place but in older anthers the tapetal nuclei divide exclusively by amitosis. Side by side with this continuous multiplication of nuclei a ~~degener~~ nuclear degeneration can be observed in which the nuclear membrane + nucleolus are resolved into a group of deeply staining fibres or narrow laminae.

These fibres are no doubt identical with

those observed by Meves in the tapetum of Nymphaea alba. These fibres have been compared by Meves to the chromidial structures found in certain ~~functionally active~~ animal cells & this comparison has a great interest in the light of Goldschmidt's recent work.

The ~~chromatin~~ fibres in the tapetum of Oenothera are found to be to a large extent derivatives of the chromatin of the nucleus & they increase in number as the cell advances in age & its nuclei continue to ~~fragment~~ divide (fragment) & to break down.

The interesting conclusion is, therefore, reached that a large portion of the material which replenishes the exhausted pollen-protoplast has at one time or another entered into the composition of a tapetal nucleus.

(9) The intine is a continuous membrane lining the entire inner surface of the

pollen grain. It first appears & reaches its greatest development at the bases of the three interstitial bodies whilst over the rest of the pollen grain it extends as an exceedingly delicate layer. During the development of the intine three thick strands of cytoplasm connect the centrally placed nucleus with the spots beneath the interstitial bodies where the membrane is growing most vigorously. In Oenothera the intine gives the reactions of a pectic body but little or no cellulose can be detected in it. In Gaura Lindheimeri & Epilobium tetragonum both pectic bodies & cellulose occur in the intine.

(10) The perforation of the closing disc of the interstitial body & the disintegration of the lamniae of that body have been most closely followed in the pollen grains of Gaura Lindheimeri. The closing disc is perforated

+ the interstitial laminae are "eaten away" ~~before the~~ in advance of the growing intine in a manner which suggests the action of a solvent, probably an enzyme.

(11) In Oenothera longiflora, even when growing under the most favorable conditions, ~~all the pollen grains do not complete their development, but many~~ many of the pollen grains become arrested in their development. They all seem to advance until their protoplast is completely exhausted by secretion but the tapetal material is insufficient to allow all of them to carry their development ~~any~~ further.

(12) The mature pollen grains are surrounded + bound together by "fibrils" which are derived from the Special-Mother-cell wall. When the Special-Mother-cell wall breaks down it forms at first a structureless mucilage which no longer gives any of the

reactions of callose. Later this mucilage becomes drawn out into "fibrils" & these are very resistant to solvents.

Rudolf Beer

Explanation of Plates

Fig 1 Lateral intercellular passage of anther of *O. biennis* in which the walls bordering upon the space here become thickened & cuticularized. Anther at stage of special-mother-cells.

Figs 2, 3 & 4 Successive stages in the development of stoma upon anther of *O. longiflora*.

Figs 5–7 Division of pollen-mother-cell showing 7 chromosomes in *O. longiflora*

Figs 8 + 9 Later stage of division of same. ~~Note crossing of fibres in Fig 8~~. All material from which Figs 5–9 were drawn was fixed with medium chrom-acetic mixture.

Figs 10 + 11. Special-mother-cells soon after completion of division. *Oenothera biennis*

Fig 12 + 13. Special-mother-cells later stage to show differentiation of the first-formed septa from the later-formed layers of the wall. Fig 12 Examined in chlorazinc-iodine solution ~~to~~ which a little phosphoric-iodine solution had been added. Fig 13. Examined in corallin soda solution ×600. *O. biennis*.

Fig 14. Raphide-Sac of anther of O. biennis. Strong Flemming's Sol + Heidenhain's haematoxylin.

Figs 15 & 16 Young pollen grain of O. biennis immediatly after formation of first pollen-wall × 860 Strong Flemming Sol.

Fig 17 Slightly older pollen-grain of O. biennis. Interstitial bodies formed × 860.

Fig 18. Similar pollen-grain to that in Fig 17 but with two nuclei

Fig 19 Pollen grain of O. biennis measuring 40 μ in diameter. Living material examined in .6% NaCl Solution.

Fig 20 Branched end of pollen-tube of Epilobium tetragonum

Figs 21 & 22 Abnormal pollen grains of O. biennis with two & one interstitial body respectively. Examined in .6% NaCl Solution

Fig 23 Pollen grain of O. biennis 45 μ across Flemming's Solution. methylene blue & fuchsin.

Fig 24 Pollen grain of O. longiflora 70μ across living material examined in .75% NaCl solution.

Fig 25 ~~Pollen grain~~ Section of pollen grain of ~~O. longiflora~~ O. biennis measuring about 86μ across the entire grain to show protoplast at end of the period of secretory activity & when it has ~~again~~ expanded inside it nearly fills the cell-cavity ∧ Flemmings Sol. Heidenhain's haematoxylin & bismarck brown

Fig 26 Similar pollen grain to Fig 25 nucleus showing first stage of mitosis

Fig 27 Section of pollen grain of O. biennis measuring about 86μ across showing Vegetative & generative (g) cells. Flemming's Sol. Heidenhain's haematoxylin & bismarck brown.

Fig 28 Pollen grain of O. biennis measuring 150μ across to show the three arms of protoplasm extending to the bases of the three interstitial bodies where the

intine was just beginning to be formed
Living material examined in .6% NaCl Solution

Fig 29 Pollen grain O. longiflora to show the three arms of cytoplasm. Fixed with Worcesters fluid. Methylene blue & fuchsin stain × 860

Fig 30 Tapetal cell of O. longiflora with 8 nuclei. Strong Flemming sol. Methylene blue + fuchsin preceded by crocein stain.

Fig 31 Tapetal cell of O. longiflora with 6 nuclei

Figs 32 – 34 Tapetal cells of O. biennis to show direct division of nucleus. Strong Flemming's Solution

Fig 35 – 39 Tapetal cells to show constricted & irregular nuclei some of which may be stages of direct division to others no such significance attaches.

Figs 40 – 42 Tapetal cells from somewhat older anthers in which pollen grains measure from 60 to 95 μ across. To show constricted nuclei.

Figs 43-45 Tapetal cells of O. longiflora to show chromidial fibres (f), degenerating nuclei (d) & intact nuclei (n). Worcesters fluid; methylene blue & fuchsin × 860

Figs 46-49 Interstitial bodies of pollen grain of Gaura Lindheimeri showing successive stages in its perforation & dissolution by the advancing intine Figs 46-48 examined in chlor-zinc-iodine solution Fig 49 stained with bismarck brown. All fixed with Flemmings Solution.

Fig 50 Interstitial body of mature pollen grain of O. longiflora. Medium chrom-acetic. Bismarck brown × 860.

Fig 51 & 52 Disintegration of special-mother-cell-wall. Oen. biennis Strong Flemming. To show the first-formed lamellae of the wall still left intact whilst the later-formed layers have completely broken down. Fig 51 Congo red stain. Fig 52 coraliin soda.