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# About the Institute

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

Ås is well known the primary sporogenons 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 fiber pollenbildende Phyllome und Kaulome. Bonn 1878. 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

In Oenothera biennis and O. longittora 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 airpassage is formed at a very early stage<sup>1</sup>). 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.

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

#### By

Rudolf Beer, Westwood, Bickley, Kent (England).

#### 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 tweetal spotted the collable Annieranni Fibrosis dec Viralistypine 1830 and since thay 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 vegetablischer 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 Ocnothera 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 sporegenous cells and an outer row of primary parietal cells<sup>4</sup>.

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

<sup>1</sup>) The terminology used here in that given in Coulter and Chamberlains "Morphology of Angiosperms" 1903, p. 33.

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-mothercells 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<sup>1</sup>.

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 carry be clearly demonstrated. The walk of the sportgenous cells and of the taperon 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 starchcontents 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 Beer, Development of the pollen grain and anther of some Onagraceae. 289

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<sup>1</sup>) 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 offici-nalis* and *Campanula rapunculoides* Mangin<sup>2</sup>) 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 of corallin in soda (4% Na<sub>2</sub>CO<sub>3</sub>), with anilme blue, benzo-purpurin or congo red, that is gives none of the cellulose reactions with Iodine reagents and is insoluble in cupranmonia, 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  $1\%_0$  caustic boots for soda. I and this strength to reduce the modulication with regard to the mother-cell wall. The mothercell wall of fresh material of Aucuba japonica dissolved with exemplary rapidity in  $1\%_0$  caustic soda but I have kept microtome sections of material of Oenothera fixed with Flemming's solution for over an hour in  $1\%_0$  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  $1\%_0$  NaOH. I have found fresh material of the pollen-mother-cells of the Horse-chestnut equally resistant to  $1\%_0$  caustic alkali. In  $10\%_0$  caustic potash the mother-cell walls of Ocnothera 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,

<sup>&</sup>lt;sup>1</sup>) 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.

<sup>&</sup>lt;sup>1</sup>) Mangin, "Observations sur la membrane du Grain de Pollen mur", (Bull, Soc, Bot. d. France, T. 36, 1889.)

<sup>&</sup>lt;sup>2</sup>) Mangin, "Observations sur le développement du pollen", (Bull. Soc. Bot. d<sub>a</sub> France. T. 36, 1889.)

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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<sup>1</sup>.

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? or pectore to account or 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 specialmother-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 longiftora 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

<sup>1</sup>) "The Histology of the sieve-tubes of Pinus". (Ann. of Bot. Vol. XV, 1901. p. 597.)

<sup>3</sup>) 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<sup>3</sup>) has called attention to three delicate lines which run through the middle of the septa of the fully grown specialmother-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 Onagraecae 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 latter stage, when the special mothercell wall on the pollen grains are therated, there handlae remain behind for some time unchanged and continue to give a very characteristic callose reaction with corallin-soda

In anthers which are a little older we observe the first appearance of the pollen membrane round each specialmother-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 calloes 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 bismark brown, fuchsin, or methylene blue; it is unstained by corallin soda, and it becomes vellow or brown in chlor-zinc-iodine solution.

<sup>1</sup>) 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 sporgenous 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<sup>1</sup> 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 callese layers and is, then alearly decognised at an independent structure. The young pollen grain by a binnity triangular, begin-shaped structure with the concavity of the "basin" directed towards the centre of the tetrad. It measures about 19 to 20  $\mu$  across its broadest surface in 0. binnis. 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  $\mu$  across in 0. *biennis*<sup>2</sup>] 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 be 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.

<sup>3</sup> 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 opposit. Beer, Development of the pollen grain and anther of some Onagraceae. 293

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 Juel1) 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

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  $\mu$  across). As was remarked above the first-formed laminae of the special-mother-cell wall maintain their individuality the longest and continue to give callest tractions for some time (Figs. 51 and 52). The toet of the wall now forms a homogeneous muchage filling the pollensac 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-zine-iodine and with a calcium-chloride solution of Iodine it tinges only yellow or yellow-brown. Congo-

 Juel, O. H., "Die Kernteilungen in den Pollenmutterzellen von Hemerocallis falva etc." (Prings. Jahrb. f. wiss. Bot. XXX. 1897, p. 205.)

Beihefte Bot. Centralbl. Bd. XIX. Abt. I. Heft 2.

<sup>&</sup>lt;sup>1</sup>) 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.)

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 Horsechestnut 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<sup>1</sup>) 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<sup>\*</sup>) 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<sup>3</sup> 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 undergoed a change and are not onger these of a pure pecie body. With follow 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 inconspicous but soon become sharp and prominent features on the membrane (Figs. 23 and 24).

In pollen grains of Oenothera longiflora 3) which measure

A 10

 After which they colour yellow to brown with Iodine in potassium odide solution.

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

a) Pollen grains measure at this time 35 to 38  $\mu$  in Oenothera biennis and O. longiflora.

•) Although I give here the actual description and measurement of the pollen of *Oenothera longiflora* the facts are essentially the same in O. biennis. Beer, Development of the pollen grain and anther of some Onagraceae. 295

from about 40 to 45  $\mu$  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<sup>1-4</sup>) 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.

Unfortunalely he gives us no details of his methods and it<sup>\*</sup> would be very desirable to know exactly what was the strength of his Guassiological 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<sup>5</sup>/, Merkel's fluid and Worcester's fluid<sup>8</sup>.

<sup>1</sup>) Fitting, H., "Ban und Entwickelungsgeschichte der Makrosporen von Isoëtes und Selaginella etc." (Bot. Zeit. Bd. 58, 1900, pp. 107-164.)

<sup>2</sup>) Denke, P., "Sporenentwickelung bei S-laginella". (Beihefte z. Bot. Centr. Bd. XII. 1992, p. 182.)

<sup>a)</sup> Lyon, M. F., "A study of the Sporangia and Gametophytes of Selaginella Apus and S. Rupestria". (Bot. Gazette Vol. XXXII. August-September 1991. pp. 124-141 and pp. 170-194.) <sup>4</sup>) Campbell, H. D., "Studies on the Gametophyte of Selaginella".

(Annals of Bot. Vol. XVI. 1902, pp. 419-428.)

<sup>b</sup>) Formulae in Chamberlains "Methods in Plant. Histology" p. 28.

<sup>6</sup>) 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.)

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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 deve-

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

			31
0,6 % Na Cl. Pollen	orain		42 11
	grain cavity		00 11,
77	cavity		30 µ,
27	protoplast	=	30 µ.
0,75 % Na Cl.			
Pollen	grain		46 µ,
	cavity		30 µ,
77	carry		00 4,
77	protoplast		30 µ.
2 % Na Cl.			

This caused complete plasmolycis. Pollen grain wavity  $26 \mu$ , protoplast = 18  $\mu$ .

4. Egg-white,

ollen	grain	-	40	JL,	
77	cavity		26	ju,	
77	protoplast		26	14,	

The results in this reagent were particularly uniform.

5. Strong Flemmings solution.

a)	Pollen	grain		42 11,
	77	cavity		28 14
	73	protoplast		26 µ.
b)	Pollen	grain	-	42 11,
	77	cavity		28 µ,
	77	protoplast		28 µ.
tron	r chro	m agotio		Intion

Gave results similar to the Flemmings solution.

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7. Merkels solution.

Pollen grain  $=40 \ \mu$  $= 30 \ \mu$ 

77	pro	toplas	t = 30	jl.
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Although difficult to recognise at this stage Merkels solution caused the protoplast to swell up and enlarge somewhat.

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II. The stamens from another bud were examined in 1. 0,75 % Na Cl. Pollen grain  $= 62 \mu \cdot 68 \mu \cdot ^{1}$ ", cavity  $= 36 \ \mu . 36 \ \mu$ ", protoplast =  $24 \mu . 30 \mu$ . 2. Egg-white. Pollen grain  $= 62 \mu . 70 \mu$ , , cavity  $= 36 \mu . 36 \mu$ ", protoplast =  $26 \mu \cdot 32 \mu$ . 3. Strong Flemmings solution. Pollen grain  $= 64 \mu.70 \mu.$ ", cavity  $= 38 \, \mu \cdot 40 \, \mu$ ", protoplast =  $26 \mu . 30 \mu$ . 4. Merkels solution. Pollen grain  $= 66 \mu$ . " cavity  $= 40 \ \mu$ . " protoplast = 40 µ. This caused the protoplast to swell up. 5. Strong chrom-acetic solution. Pollen grain  $= 66 \mu$ . " cavity  $= 36 \mu$ . " protoplast =  $28 \mu$ . III. Stamens from another bud examined in 1. 0.75 % Na Cl. Call cavity O 46 k m result of the second second2. Examined in a drop of juice squeezed from the stem of Oenothera longiflora. Pollen grain  $= 76 \mu . 76 \mu . 80 \mu$ . ", cavity =  $42 \mu \cdot 44 \mu \cdot 46 \mu$ . ", protoplast =  $32 \mu . 36 \mu . 34 \mu$ . 3. In 5% cane-sugar solution. Pollen grain  $= 72 \mu$ , cavity  $= 42 \mu$ , ", protoplast =  $30 \mu$ . IV. In another bud pollen examined in a drop squeezed from stem of Oenothera longiflora. Pollen grain  $= 72 \mu$ , , cavity  $= 42 \mu$ , " protoplast = 34 µ. V. In another bud pollen was examined in Pollen grain  $= 62 \mu$ , cavity  $= 34 \mu$ ,  $protoplast = 28 \mu$ .

<sup>1</sup>) The successive numbers (62  $\mu$  and 68  $\mu$  in this case) denote measurements of several pollen grains. Each vertical series of figures corresponds to one pollen grain.

2. Absolute Alcohol.

Pollen grain  $= 62 \mu$ " 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  $= 68 \mu$ .

" cavity 
$$= 41 \mu$$

", protoplast =  $28 \mu$ .

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

4. In 5% cane sugar.

28 to 30 / 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 u and was obviously

The following observations upon O. biennis may also be

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 % NaCl solution to the above, watching the effect all the time. The pollen grains remained unchanged both in their appearance and in their measure-

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:

> = 74 u. " cavity  $= 40 \mu$ ,  $protoplast = 30 \mu$

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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 % NaCl, 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
$62 \mu$	$37 \mu$	27 µ
70 µ	39 µ	$32 \mu$
$74 \mu$	$\pm 2 \mu$	$34.25 \mu$
80 µ	46 µ	34.5 //.

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

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

Distilled water onlarges the vacuales and causes them to run together by breaking down the separating arms and faminae of cytoplasm. Consequently the whole protoplast swells up

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 % NaCl 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:

ollen	grain	74	11,
77		40	
77	protoplast	30	ļl.

2. Sections of anther of about same age fixed in Flemmings solution:

 $\begin{array}{rl} \text{ollen grain} &= 72 \ \mu \, . \, 70 \ \mu \, . \, 40 \$ 

 $protoplast = 26 \mu . 28 \mu . 24 \mu . 22 \mu.$ 

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 source from which the plastic material of the 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 h

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

<sup>1</sup>) Strasburger in his work of 1882 already wrote of *Gaura biennis*. "In meinen Alkoholpröparaten bildet der nach Anlage der Wand erschöpfte Inhalt der Pollenzelle nur noch ein unscheinbares Klümpchen" cf. his Figs. 47, 48 and 49, Tafel VL. Beer, Development of the pollen grain and anther of some Onagraceae. 301

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<sup>4</sup>) the first wall appears as a single homogeneous lamella but when the grain has grown and measures about 40  $\mu$  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  $\mu$ ) its first membrane can be clearly seen to consist of a thin, outer homogeneous layer and an inner "rodlet" layer (Stäbchenschicht or Anschlußlamella<sup>2</sup>.

The growth of this membrane recalls Strasburger's<sup>3</sup>) description of the first pollen-wall of *Althace 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 *Althace*.

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  $\mu$  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 onter 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.

<sup>1</sup>) 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.

<sup>2</sup>) Strasburger, "Die pflanzlichen Zellhäute". (Pringsh. Jahrb. f. wiss Bot. Bd. XXXI. 1898, p. 551.)

<sup>3</sup>) l. c. p. 555.

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The interstitial bodies are shut off from the eavity 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 onter 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  $\mu$  and 95  $\mu$  in both 0. biennis and 0. longitora 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 unphases of mitorit division Fig. 26). It have not followed the datalk of this division where lease 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)

The tapetum now breaks down and its contents clearly furnish the material for the renewed growth of the pollenprotonlasts

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<sup>1</sup> 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 *Deroblera* after the appearance of the first pollen wall so that it 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.

<sup>1</sup>) 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  $M eves^{1}$  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,<sup>2</sup>) 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 chather some of them are lost sight of in the course of further changes. I an anable 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,<sup>8</sup>) 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.

<sup>3</sup>) Which are two or three in number.

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The comparison which Meyes 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, yolknuclei, chromidien, apparato reticolare etc.

Goldschmidt<sup>1</sup>) 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.<sup>2</sup>) 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 Golds-Cohmid it is inghly probable that they are derived from the original statements.

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

<sup>1</sup> Goldschmidt, R. "Der Chromidialapparat lebhaft funktionierender Gewebzellen". (Zoolog, Jahrb. Abt. f. Anat. u. Ontogenie d. Tiere. Bd, XXI, 1904. p. p. 1–100)

<sup>2</sup>) 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. 1859.)

<sup>&</sup>lt;sup>1</sup>) M e v e s, Fr. "Über das Vorkommen von Mitochondrien bezw. Chondromitten in Pflanzenzellen." (Berichte d. Deutsch. bot. Gesell. XXII 1904, pp. 284-286.)

<sup>2)</sup> Wor cester's fluid is by far the best fixatine for this purpose.

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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, enticularised 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 intime 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 an interest.

. 1.

In still older pollen-grains, measuring from 108 to  $112 \mu$  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 *Ocuothera* at a corresponding age. In older grains this structureless mucilage becomes distinctly laminated. These laminae are very closely arranged at the base of the interstinial body and form there a closing disc.<sup>1</sup>)

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 oft and even much ginous 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 longiftora* measures between 170 and 180  $\mu$  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  $\mu$  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 callese dyes and have become very ensistent toy-solvents. Their producties in many respects resemble those of outculausied structures.

In the species of *Epitobium* 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 cellmembranes 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-mothercell 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 irreBeer, Development of the pollen grain and anther of some Onagraceae. 309

gularities 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.

 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  $\mu$  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  $\mu$ to about 46  $\mu$ . The protoplast, however, grows far less rapidly during this time and its diameter only enlarges from 26  $\mu$  to about 34  $\mu$ 

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

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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

8 111 The material required for the growth of the membranes is derived from the tecretory activity of the pollen-proto-

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<sup>1</sup>) 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 Nymphaca 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 pollenprotoplast 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 extoplasm connect the centrally placed muchus with the spots beneath the interstitial bidders where the membrane is growing most vigoously. In *Oenothera* the intine gives the reactions of a peetic body but little or no cellulose can be detected in it. In *Gaura Lindheimeri* and *Epilabium tetragonum* both peetic 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 *Gauera 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-mothercell 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.

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### Explanation of Plates 3, 4 and 5.

- 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
- 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
- Fig. 10 and 11. Special-mother-cells soon after completion of division. Ocnothera
- 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.
- Raphide-sac of anther of O. biennis, Strong Flemming's sol. Fig. 14. 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.
- Slightly older pollen-grain of O. biennis. Interstitial bodies
- Similar pollen-grain to that in Fig. 17 but with two nuclei. Pollen grain of O. biennis measuring 40 µ in diameter. Living Fig. 19. material examined in . 6% Na Cl solution.
- Branched end of pollen-tube of Epilobium tetragonum.
- Fig. 20. Fig. 21 and 22. Abnormal pollen grains of O. biennis with two and one inter-
- stitial body respectively. Examined in . 6% Na Cl solution.
- Fig. 28.

Fig. 24.

mined in . 75 % Na Cl solution.

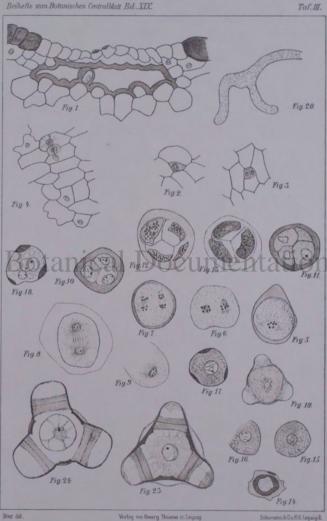
- Section of pollen grain of O. biennis measuring about 86 µ Fig. 25. across the entire grain to show protoplast at end of the period of secretory activity and when it has expanded until it nearly fills the cell-cavity Flemming's sol. Heidenhain's
- Similar pollen grain to Fig. 25 nucleus showing first stage of Fig. 26.
- Section of pollen grain of O. biennis measuring about 86  $\mu$ across showing vegetative and generative (g) cells. Flemming's sol. Heidenhein's haematoxylin and bismarck brown.
- 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.
- plasm. Fixed with Worcester's fluid. Methylene blue and
- Tapetal cell of O. longiflora with 8 nuclei. Strong Flemming Fig. 30. sol. Methylene blue and fuchsin preceded by crocein stain.
- Tapetal cell of O. longiflora with 6 nuclei.
- Tapetal cells of O. biennis to show direct division of nucleus. Fig. 32-84.
- Fig. 35-39. Tapetal cells to show constricted and irregular nuclei some
- Fig. 40-42. Tapetal cells from somewhat older anthers in which pollen

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- 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 Lindhcimeri showing successive stages in its perforation and dissolution. by the advancing intine Fig. 46-48 examined in chlor-zinciodine 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. Ocn. 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.

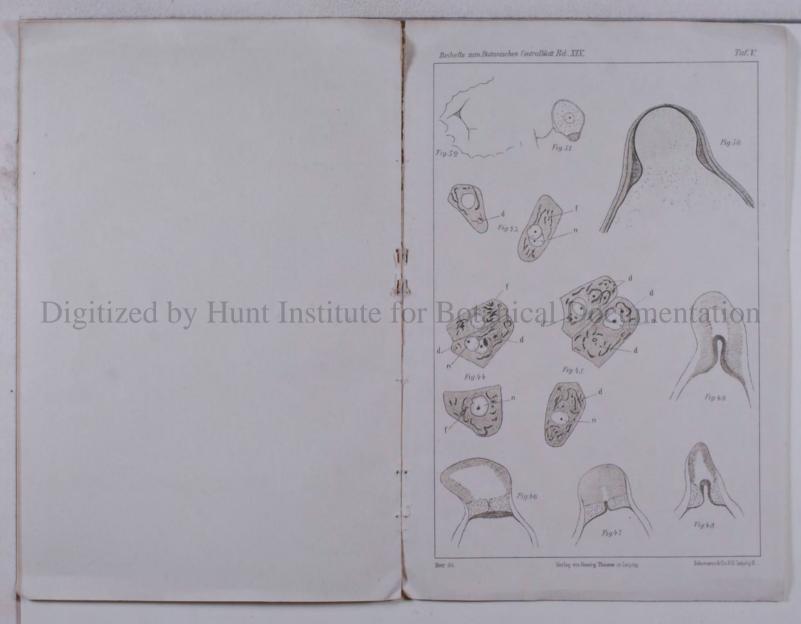
## Pollen grain of D. busines to u across Flemming s-solution-methylene Jud and fuchsin. Pollen grain $\mathcal{G}$ . long flora $70~\mu$ across. Living material exafor Botanical Documentation

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Taf.III.





Bertelle "Erste Ablheilung" ca 1 4 200 B5a On the development of the pollen grain + author of some magraceae Rudolf Deer, Westwood, Dickley, Kent ( Singland ) Mit 3 Tafiling the pollen grains of the Inagraceae has altracted the allention of bolanists from a very sarly date. Already in 1830 Purkinje Examined o figured the pollen of Several Species ( De cellalis antherarum "Fibrosis ste" Vratislevice Digitized by Hunt institute tor Botan Hals Documentation Fitsche, Schacht, Nägeli, Luerssen, Is chistickoff, Sachs, Wille + Stresburger, as well as others tiene all paid greater or less allention to this subject. By far the most detailed account which we possess is that of Strasburger embodied in his two memoirs upon the cell - walk (1) heber den Bau und das Wachsthum der Lelkäute 1882 pp 95-100. (2) Uber das Wachsthum Vegetabilischer 1889 pp. 36 - 46) Lellhaute

- 2 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 Denothera longiflora, O. biennis + Gaura Lindheimeri Epilobium tetragonum + E. montanum have also been examined but less this thoroughly . Dignized by Hunt Institute for Botanical Doctanes pation in quite the usual manner. by the division of a single longitudinal row of typodernal cells ( the or chesporum) divide into an inner Series of primary sporogenous cells + an onter row of primary parietal cells." on the latter a duccession of periclinal divisions follow one another until resuely, four layers of cells separate the column of primary sporogenous (1) The terminology used here is that given in Coulter + Chamberlain's " Morphology of angeosperus" 1903 p.33.

cells from the epidermis. The outermost parcetal layer is the endattee cium or fibrons layer; within this follow (usually) two "middle layers" + finally, adjoining the sporogenous tessue, the tapetum becomes differentiated. les is well known the primary sporogenous cells of Saura form a single longitudinal row. Subsequently certain of these sporogenous cells become "sterile" +, by their division, form transverse Septa here & there, along the author tized by Hunt Institute for Botanical Documentation the magraceae has already been described by Barcianu (Inang. Diss. Deipzig 1844 " Unters. über die Blüttentwick. d. Duagraceae p. 21) + by Bower (Studies in the Morphology of Spore - producing members . Il Ophioglossaceae 1896 p.1) Large raphide - saes seens in the connective of all the species examined. The bundle of orystel of Each Sac is Enveloped in a mucelage

which stains violet with a mixture of methylene blue + fuchsin , + peak with rutherium red . These reactions indicate a pectic body. The crystals + thur muchage sheath do not fill the suture Sac but the space which is left between them I the wall of the Sac is over occupied by a material which Les after tos a reticulate structure . In Heidenhains Dron hæmatorglin the muilage Sheath becomes black Whilst the reticular investment remains uncoloured This sale retracte male Substance is probably Digitized by Hun Woitstar for Botabic Alestiden Sunna Been in the Warning in his description of the anther of Epilobium angustifolium (Unters. meber pollen bildende Phyllome & Raulome. 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 and in the species of Epilobian.

In Denottera beennis & O. longiflora tannin also occurs in the anther, both in the upidomal epidernies & in a Varying number of cells of the connective. In 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, tennin-free bands soon ceases to Stow + become stretched & flattened by the enlargement of the anther. Beneath each of these Digitized by Hart pe culiar epidermal cells a longdime longitudinal air-passage is formed at a very early stage . 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.

I dome anthers a currons development of the cell-wells bordering upon the air-passage was abserved. In the cases the The cell-wells in question had become greatly theckened retime Spat monoraly So, & cute calarised in a menner which was quete Similar to that found in the wells of the pollen gracens themselves . The air-passage in these anthers was therefore completely shut off by a continuous mentle of the ck, cutenesed membranes. The thecking Digitized by Hunt Institute for Botanica had cathen place Very early in the history of the author long before the pollen wells themselves had undergone theme Such changes & and indeed before the pollen- well had put in its appearance at all. (Fig. 1) Stomata occur upon the anther but they are not very abundant. In anothers 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 the certain of the merislematic superficial cells of the anther F this becomes the direct matter - cell of the Stoma & Starch can nearly always be found in the guard cells of the Stoma although the atter epidermal cells are quite free from this Substance." I have examined the anthers of & gava Lindheimere soon after the primary sporogenous cells have become definitely established by means of the cell-walk reagents recommended by Mangin. Dightized by Hunt Institute for Establical Documentation are present in the walls of the author - cells at this time but that the cellulore is ordinarily masked by the pectre constituent. It is only after breaking the Sections with delute aced followed by the action of delate alkali the the cellulose can be clearly demonstrated. The wells of the Sporagenous cells & of the taketum contain less cellulore then the other regions of the anther.

(1) a little Starch occasionally occurs in the epidermal cells of the connective fast over the Vescular bundle, but never in at any other part of the epidermis.

The walls of the primary sporagenous cells are at first no thicker than those of the surrounding tessues but they Soon increase in thickness + stand out conspicuously from the neighbouring membranes. The very young aultur contains only a trace of starch in fine granules. The occurrence of starch can first be detected in the filement of the Stemen, it then spreads upwards to the cells of the connection which he dorsal to the vascular bundle & it can next be seen in the primary sporagenous cells. This is the usual Digitizadoby Hapt beatizete taxabatanical Documentations 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 Saura the sengle longitudinal series of primary sporagenous cells

& Spara the princip opprogenous all , shick have been cut off from the hypotomak cells, becomposed, without any further congitudinal division cells sach avrounded by a well of peculiar nature. the primary Sporogenous cells undergo a rengle Confitudenal devision so tot two rows of mother - cells are formed whilst a Denothera a second longitudinal wall after follows the forst So that retter two on three mother-cells are seen in the transverse Section of Each pollen sac The reat important step in development is the formation of a muchagenous make well round Each matter-cell. From to foret appearance this walk gives none of the reactions y seller cellatore save or ap pectore but its which Mangin has shown to be characteristic of callon,

10 This wall is smentrally Similar to that which occurs in a seen corresponding position in other angiosperms. Mangin examined the mother-- cell walls af an a number of flowering plants + concluded, from their mecrochemical behaviour that they consist of callose in a pecalierly pure state. & In Gentiena officinalis & Campanula rapunculoides Mangin hated some Variations in the composition of the som (special-) mother - cell well. Digtized der Hants I chate tutte for Bertanised in Schere feit at ditte a Solution of corallin in Soda (4% Na Co3), with ancline blue, benzo-purpurin or congo red, that it genes none of the cellabore reactions with Lodine reagents + is insoluble in cuprammonia, & that it has no affinity for ruthenium red is a gree with Mangin in considering callose to his only constituents the the track. "Observations sur la membrane du Grain de (1) mangin Pollen mur" Bull. Soc. Bot. d. France 7:36 "Observations sur le déceloppement du pollen." (2) 11 Ball. Soc. Bat. d. France T 36 . 1889

In several respects, however, I find the reactions of the matter-cell walk to disagree from those usually attributed to callor Callose is described as readily soluble in 1% caustic potase or soda. I find this statement to require some modification with regard to the matter - cell wall. The (Special) matter-cell well of prosh material of Aucuba japonica dissolved with exemplary rapidity in 1% caustic soda but I have kept succratione Sections of malenel of Denothera fixed with Hemmings Solution for over an hour in Digitized bautint poteste for Stillen found to masterion cells undiscolved at the end of that time. The matter-cell walls of fresh material of O. beaunis had only disappeared after nearly 24 hours in 1% NaO4 I have found fresh material of the pollenmother - cells af the Horse-chestnat squally resistant to 1% caustic alkali. In 10% caustic potash my ten mather-cell walls of Ornothera Soon desappear. It will be seen from these remarks that there is some Variation in the Solubility of the matter - cell walk in delate caustic alkales

mangin has afformed tail callose is Solible in phosphoric acide but I have left the mother - cell walls of Denothera biennis for many hours in strong phospharin acid without obtaining any signs of this developing. Naphtal black, a loss said by mangin to Stain cellulose but to leave callose uncoloning but I have obtained precisely the opposite resalt. I have sitter an aind on a reative Solution of happeter black Digit/sed by Hunbrystitutentery Batanical Docymaniation all described as pectic stains which leave callose uncoloured. I have found them stain the mother-cell walls the although not 's deeply as the pectre walls membranes.

The origen of callose has formed the subject of repeated descussion. In the case of the callor of the Seene - tubes some have addented that this Substance areas from the transformation of pre-easting cellulore, whilst others believe it to be a direct product of protoplasmic activity orgenating without any relation to cellabore or atter fore runner. Hell in tis account of the Seene - takes of Pences, believes that callose may originate sometimes directly t Digitized By Hand Institute for Botanical Documentation In the case of the callose composing the pollen mother-cell walls there can be no doubt of concerning its mode of oregen. It has already been mentioned tal in the very young an thes the walls of the primary Sporogenous cells are poorer on cellulor them the other tessues of the anther, on Somewhat older anthers, the long before the matter-cell "The Histology of the Sieve tubes of Pinus" ann of Bot. Val XV 1901 p. 599

Thoreown the cellulose which occurs in the young ball is morely a trace + could not give nice to the massime callose walk

Digitized by Hunt Institute for Botanical Documentation

wall may be espected to appear, the membranes of the sporagenous cells no longer show any traces of cellulore but this, if it still occurs (which is doubtful), is completely masked by the pecter bodies which are Strikingly developed in these membranes. Even after breatment with delate aced + alkali - as recommended by mangin-I was unable to fend as demonstrale any celluloze in these walls. It is within these walls that the callose gized by is developed its for Botanical Doctomentarion lager makes it appearance () disappearance of Setter cellulose or pectore to account for a transformation of these Substances into Callore. Whateau 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 calloan is derived directly from the activity of the proloplast without the intermediation of cellulose (1) The cellulore is lost right of in the walls of the sporogenous cells too long before the callore appears for there to the have any due connection with one another.

15 Zach matter - cell now divides to form the four Special - mother - cells. The milatic figure is rather small + not well adapted for studging the details of nuclear division. I will content myself, therefore, with stating Ital in Densetura longiflora the number of chromosomes which appear at the first of Second devicions of the pollen moltur-cell is Seven. They are so Small in size that I distinguish them as Somewhat Digitizegular Hyranules the foolether they have a definite I constant shape pecalion to Each division (as Seems likely) could not be certainly determined. A the Somatic divisions ( which I have studied in the wall-cells of the anther) the chromosomes have the form of curved rods which are in crowded together upon the spindle "that it is not defforable to count them . I have made out thaten the ter humber will probably prove to be fourteen. I have made out distinguished 13 to 14 in some cases + the latter number will probably prove to be the correct on the

16 Between the cells of stetrad, which results from Matter cell tout. this division. Septa are developed which form an satension of the mucilegenous mother-cell wall. Like the latter these septer also que the reactions of callose. (Figs 10 + 11) mangin has called attention to three delicate lines which run through the contra middle of the septa of the fully grow speciel mother all well the centre of the tetred, in the case of alther roses. Dig Wate pointed water state to the Beating icher of the offer on granular in Structure + he believed them to be retrogenous in hature. Other authors have fegured these radiating lines a & the Special - mother - cells of atter plants; Strasburger figuring them both for althaca rosea + Gaura biennis as long ago as 1882. I have observed these lines in all the V) Bull. Soc. Bot. d. France T. 36. 1889. p. 391 Mangin described the special-mother- cell well of althave rodea .

Onagraceae which I have examined. I ty careful focussing + by the comparison of # series of "microtome sections I find these lines to be the aptical repression of laminae. Most probably these lamenae represent the first lamellae deposited after the completion of cell division. They believe Somewhat differently towards reagents They differ Somewhat in their behaviour towards Stains but their reactions still indicate their (Figs 12+13) at a later stare, Callose composition . Moreover, when the Dispized by Flat Instituter of the Potatical ko achimentation believe for some time + continue to five a Very cheractoristic N unchanged + continue to five a Very cheractoristic callose reaction with corellin - Soda white the rest of the well which has disintegrated ho longer tas 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.

18 We first recognise it as a very delicate film levering Each cell - cavity of the tetrad . It is in most intimate contact with the callose well + even reagent which cause general platmolysis + considerable distortion of the & cell-wells of the ancher Seldom reparate the very young pallen wentrese from the spicel-matter-cell wall. The protoplast of ten cell is also sand firmly attached to the new membrane, but it is Easier to tear away the cytoplasm from it than it is Digitized by and I lastitute for Botanical Docementation From the first, however, it can be distinguished from the special-mother-call well by its behavior. towns & reagents. It steens red with ruthenium red ; it colours much more deeply than the section well with biomarch brown, fuchsing or methylene blue; it is unsteened by corellin Soda, title comes yellow or brown in theory - todane Chlor- Zene - rodene Solution . When apose tetrades at this stage are breaked

X

with 10% KOH. the of calloace well is devolved + the protoplasts, Each surrounded by the young pollen walk membrane, are set free. I peripheral lagor of the tetrad behaving Sundas alltrogh the We may conclude from these reactions that the young pallen wall is composed of a pectie substance. The remains of the primery Sporogenous cell well, which the also of a afa perter court gives the reactions af pectic body Can be destinguished tat the aportphory of the lebrad although the association between the newly dealloped pollen membrane & the Special-mother - cell wall is so close the demarcation between the two is always sharp I there is here a gradation of one ento the atter. Where the callose well abouts upon the pecter membrane it is denser & more togation a appearance refracts the light more strongly them the rest of tuball probably forming a grenzhautchen in Strasburgers sense

The facts show that although the pollen walk is at first deposited in close contact with , + probably in actual atlachment to, the special - moltion - cell ball it is not dereved from a transformation of the unermost lamellas of this callose wall but is directly sente secreted as a pecter layer by the cell-protoplast. It is Equally certain that the plasmoderma" is not body transformed into the pollen walk 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 tacker it separates readily from the callose layers & is then clearly re agrissed as an independent structure the young pollen grain is a bluntly triangular, basin shaped structure with the conceptity of the basin derected towards the centre of the tetrad. It measures about 19 to 20pe across its broadest Surface in O. beennis. At the apex of the three angles of the pollen grain the well is satrenely then. The protoplasm fills the cell-cavity & contains a considerate quartity of Starch . (Figs 154-16) on pollen grains which are a lettle older (1) I use this term as the equivalent of the german "Hautschicht". "He word was for saggested for this purpose by Strasburger + first used by Stevens in his paper upon " Gametasenesis & Fertilization in albugo" Bat. Gayette XXXII 1901 p.92.

21 in O. beannies (meaning about 22 to 24 pe across) the wall has theckened considerably + a muchagenons material has been developed at the three angles of the cell at those spots which at the present Since the reactions of a pectic Substance & alteration a of the pollen well at these points . The little plugs or discs of mullage continue to enlarge & soon balge so far within the cell Digitied they overlap the or Botanical DosuBentation on Each Side. at front the machine of Tax mailignous alles The name of "Zwischenkorper", introduced by Fritsche, has been used by Nageli + Strasburger in describing these pecaliar muclesinons desces of the Onagraceons pollen grain. I Shall speak of these discs as "interstitial bodies" in the present paper. In the large maporly Three is the hormal 13 In getating the massificate of the pallen grain I have always taken the measurement a cross the broad face of the grain from the tip of an interstitive body to the outer Surface of the walk immediatly appointe.

number of interstitial bodies possessed by the pollen grains of all the species of magraceae 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 interstitual 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 Digiprobably Honkencomparia Bulknitte Diragularitieson which fuel "& others have described in the nuclear divesion of the pollen - mother - cells of Hemoro calles fulva & 15 ho doubt due to one or more chromosomes becoming deparated from turest & 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) Jul O.H "Die Kerntheilungen in den Pollenmutter zellen von Hemero callis fulva rtc" Prings. Jahrb. f. 10iss. Bot XXX 1894 p.205

a large number of my preparations of Den. biennis Show this to occur at the comparatual sarly age that we are now considering (Viz: pallen 22-24 je across). As was remarked above the first - formed lamenae of the Special - matter -cell wall maintain their integral individuality the longest + continue to give callose reactions for some time A The rest of the walk now forms a homogeneous mucilage filling the pollen - Sac + occupying all the spaces Digitized by Hust Institute for Botanical Documentation has any affinity for corallin- Soda + 05 reactions furnish no clue to its chemical nature. Us the pollen grains continue to develope the interstitial badies become more prominent broader face of the towards the raterior, giving the grain a more pronounced triangular ontline.

a secondary theckening layer is now dapas formed within the forst pollen wall. This

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This layer estends over the whole uner face of the forst membrane of the pollen grain. It runs up the deckes of each interstitual body as a cylindrical Estension which gradually thus off as it approaches the apen of the body of dies away altogether at the summet itself & The alamased microchemical reactions of the theckening layer do not correspond with the which are characterestic of any of the ordinary cell-wall components + its chemical hature Digitized by for the notifie tor Bokapic an Documentation With a rather Strong Solution of Lodine in potasseum codide it genes a very beautiful / Violet colows but with chlor - Zine - eadine + with a calcum - chloride Solution of Indine it tinges only fintly yellow Congo-red leaves it unstained. Pollen grains which there been Stained with a read methy sene - blue & fuchsin I have found that the layer pink or violet. The first pollen - walk of the Horse - cheshuel

24

in its Early stages, gives reactions which are almost identical with those of the Secondary layer of Denothera apart from the very Striking Violet reaction with the Lodine Solution the properties of these membranes correspond fairly well with those characteristic of fectic Substances & it is not improbable that we are here dealing with an association between a pectre nature ... body + a substance of unknown composition Additional Support 15 geven to this view by the fact that the Violet reaction becomes lost after Digitization of Infunction spiteten for Bataniciet Das water taking no doubt because the habet body which gives the reaction is soluble in alcohol. In its beheavour towards other reagents, however, the thickening layer remains unaltered after an enmersion in alcohol. Cuticalarisation takes place very Early in these membranes to the Violet-reacting body may be associated with the fust stages of this process.

" after which they & colour yellow to brown with Sodine is potassein indide solution.

25

26 In alcohol material the thickening layer, at the Early periods of its development, is after greatly Swallen" + this becomes more marked + may soon lead to the bursting of the pollen grains of this is Samuned in aqueous Solutions. The interstitual body is now polespin according 36 to 38 pr i Dea bennis & Den Congiflara) limited towards the cavity of the grain by a closing dese which has the same composition as the rest of that body although it is smealet Digitized by Hunt Institut the Botanical Documentation enterstated body have undergone a change + are no longer those of a fure pectic body. With I adine reagents it colours yellow; with Congo red it stains uniformly red; with haphtal black it colours blue - black; with negrosin it becomes black; with rulkenium red it been Steens red; with methylene blue - fucksin mexture it colours blue, pink or violet according to the strength of the solution used ; with corallin as Pollen grains measure at this time 35 to 38 fe in Oensettura biennis + O. longiflora z (1) This was already noticed by Strasburger in Gaura biennis

24 -Soda solution it remains colourless. The protoplast fills the cevily of the pollen grain at this stage but weak plasmoly sing agents show that, whilst it is firmly fraed to the developing Deconderry layer, it is free from the bases of the interstitual bodies. Us the thickening layer of the pollen walk continues its development ring-shaped ridges make their appearance at the bases of the interstitial bodies. These are at first low + Digitized by Hunt Institutestor Batanical Docymentation features on the membrane. (718023+24) In pollen grains of Denathera longiflora which meadure from about 40 pe to 45 pe the protaplast Still completely fills the cell - cevity but has become Entirely free from its walls. The diameter of the paller Sam non rapidly enlarges The further increase in the size of the paleen Stain , now takes place , more rapidly them (1) although I give here the actual description & measurement of the pollen of Denschere Congiflore the facts are essentially the Same in O. beennis. Attendescent

28 that of the leving protoplast which we cell-centry high We here field the conditions which strikingly recall those Which Fitting & others have described in the case of the megaspores of Isoeles + Jelaginella these results have such an emportant bearing upon our conceptions of the growth of Régétable membranes for Botander Documentation features of this growthe process so difficult There resitated to accept them until they could be placed upon a broader basis them (1) Fitting H. Ban a Entrickelangsgeschickte der Makrosporen von Imitee + Selazinella ste "Bot. Zeit · 1900 Bd 58 pp. 107-164. (2) Denke P. "Sporenentwickeling bei Selagenella" Beihefte 2. Bat. Centr. Bd XII 1902 p.182 (3) Lyon M. F. a Study of the Sporangia + Gemetophytes of Selaginella apus + S! Rupestris". Bot - Gagette Vol XXXII august - September 1901 pp 124 - 141 + pp. 170 - 194 (4) Campbell. H.D. " Studies on the Gametophyte of "Selaginella" annals of Bat. Vol XVI 1902 pp. 419 - 428.

has done by those who have examined the megaspores of the dycapodiales. With the exception of Filting, these authors have exclusively realed then conclusions upon mecrotome sections. Invaluable as Such sections are we must not overlook the fact that the long series of manipulations necessary for killing, fixing + embedding in passa paraffer introduce many possible Sources of error & the results obtained by this stized by the stitute fully checked by obtervations upon leving material. Filting worked largely with living spores which he examined partly in phy scological Salt Solution I partly in water. The latter is unquestically and insistable meduum for the week. Unfortunally he gives us no details of his methods observations + it would be very descrable to know exactly what strength of his physiological Salt Solution to to whether this particular concentration was found by direct raperiment

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30 to produce less change in the cell than, other strengthy. Weter was a work ansaitelde His selection of water as an alternative medium in which to examine the condition of the protoplast bes most unsuitable to no religne con the indergramper to affect the protoplasm & its plasmo obmatic condition. in a large humber of cades. The poleen grains of Denothera' are particularly favourable for investigation + I have altempted to make buy shawin alton of them taisn Complete as possible. Fresh material has been examined in the forst place + the results this obtained have been compared with me crotome sections of material fixed with strong + weak Flemming's Solutions, with Strong & medeum chrom-acetic Solution, merkels fluid + Worcester's fluid. The succrotome Sections were particularly useful (1) Formulae in Chamberlain's " methods in Plant Histology" (2) Formula for this fluid was obtained from H.S. Read's paper upon Engrand Secreting cells of Lea + Phoenix. ann. Bot. april 1904 p. 271

in showing the exact relations which exists between the pollen grains + the alter cells after anther at the defferent periods of development. I will give here a few of the measurments which I have made of the pollen grain, its cell-cevity + its protoplast. The stemens were examined directly after the removal of the flower buds from the plant's which were all strong the althy plants growing upon an apen plat of ground. The pallen grains were Digitized axed wet a net tute for Botenical Accumentation drop of the fluid which was being studied + rapidly ramined whilst still uncovered. 1. The stemens from one bud were successively examined in the following solutions ( . longiflora). (1) . 6% Nace Pollengrain = 42 p pollen cenity = 30 p pollen protoplast = 30 p · 75% Nace Pollengrain = 46 pe " cavity = 30 pe " protoplast = 30 pe

32 (3) 2% Nace This caused complete plasmolyces Pallen grain = 40 pc " cavity = 26 pc " protoplast = 18 pc (4) Egg- white Pollen - grain = 40 pc " cavity = 26 pc " protoplast = 26 p The results in this reagent were particularly uniform. Digitized by Store In Frence for Botatical Documentation (a) Pollen grain = 42 pc " cevity = 28 fe " protoplast = 26 fr Pallen grain = 42 p (6) " cavity = 28 fr " protoplast = 28 pc (6) Medium chrom-acetic Coursed Slight more Shrinkage them the Hamming's sol. Pollon grain = 44 h " parity = 30 h " protopliot = 20 fe

33 (6) Strong chrom a cetic solution Gene results Similar to the Flemming's Solution ( ) 189 merkel's solution Pallen grain = 40 pc " county = 30 h . protoplast = 30 h although difficult to recognize at this stage merkel's sal caused the protoplast to swell up & enlarge Somewhat. Diffuzed by Heat Institute for Batanical Documentation Examined in (1) . 75%. Nace Pollen grain = 62 / . top. 68/ " cavity = 36 pm , 36 pm . 36 pm n protoplast = 24 / . Som. 30/ (2) Egg-white Pollen grain = 62 pc. 200 . 70 pc " cavity = 

34 (3) Strong Flemming's Solution Pollen grain = 64 p. 70 h .. cavity = 38 pc. 40 pc " protoplast = 26 fr. 30 pc (4) Merkel's Solution Pollen grain = 66 fe . cavity = 40 fe " protoplast = 40 h Yhis caused the protopeast to Swell up. Digitized by Hunt Institute for Botanical Document (5) Strong Chrom - a celic Solution Pollen grain = 66 h " cavity = 36 fr " protoplast = 28 h Itemens from another bud samined in TT (1) .75%. Nace Pollen from = 80 p . canety = 46 pc " protoplast = 32/

35 (2) Exemmed in a drop of fince Squeezed from the Stem of Densteina longiflora. Pallen grain = 76 p. 76 p. 80 p .. carity = 42 p. . 44 p. . 46 p. " protoplast = 32 p. 36 p. 34 p. (3) In 5% cane - Sugar Solution Pollen grein = 72 fr " cavity = 42 p . protoplast = 30 p. Divitized by Launt Anstituts for Bot miled Decumentation a drop Squeezed fro Stem of Denath. Ingeflore: Pollen grain = 72 fr " carity = 42 p V. In analter bud was examined in :-(1) · 6% Nace Pollen grain = 62 fr · cavity = 34 h " protoplant = 28 fe

36 (2) Absolute alcohol Pallen grain = 62 p polan cavity = not measured " protoplast = 16 pm alcohol always caused great shrinkage of the protoplast, the distortion of the pollen menterene. VI The following observations were made upon the pollen grains in the following media: -(1) The Slamen was placed in a drap of Digitized by Huer Institute for Boateral Decenterion out without coming into contact with the air. Pallen grain = 68 pc " cavity = 41 p " protoplast = 28 pm (2) Pollen grains teased rapidly into distilled water & ammediatly Examined showed a protoplast of 28 p in pollen grains 68 per a cross; Very Soon hoven the Vacuoles of the protoplast sularged ruptured the separatures arms & leminee of cytopleron + ran together and so

34 that the whole protoplast Slowly Swelled up until it quite filled the pollen - carity (42 h). (3) In 2% cane sugar the results were Similar to those an distilled water. (4) a 5% cane - Ingar The protoplast for some time maintained ets Size of 28 to 30 p. Nater, homen in some of the pollen grains changes Similar to those of (2) + (3) were seen but much less marked. Digitized by Hunt Institute for Botanical Documentation The protoplast measured about 28p + it remained unaltered after a prolonged examination (6) & 2%. Nace Protoplast measured 224 the only 20 for & was obviously plasmoly sed. The following observations upon O. biennis may also be mentioned.

38 as possible I carefully I as rapidly teased out the pollen grains into a lettle of the mucilaginous fluid which can be Squeezed from the anether itself. In a pollen grain measuring 68 p + a cavity of about 40 p the polten grain protoplast measured 30 pm. I then added a drop of . 6%. Nach Solution to the above, + again measured the poleon grain a the protoplant watching watching the effect all the time. The pollen Digitized byr Hunst Institute for Botapical Doctimentation appearance + in their measurements. In another Similar raperiment upon another bad from a different plant the measurements bath in the anther-price & is the . 6% Nace Solution bere:-Pollen grain = 74/ " cavity = 40 p " protoplast = 30 pm

39 I meght occupy many pages in quoting Similar measurements but as those which I have mentioned 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% Nace, . 75% Nace, 6% can-- Sugar, & egg-white + the plants own juice the protoplast has a Very Similar appearance + its measurements agree very well with one another at the Moreour, after remaining in these Solutions for sme Digitized back Pupt Institute for Botanicate Dacumentation appearance, was observable. The average measurement in these Solutions calculated from all my holes are as follows :-Pollen grain pallen cavity protaplast 40 p 26 pc 26 pc 62 p 27 / 37 K 32 p 70 ju 39 / 74 10 42 pc 34.25 je 46 p 80 pc 34.5 ju

Strong Flemming's Solution + Strong chrom-acetic Solution do not alter the protoplast very much in appearance but usually cause Some Shrinkage. merkels fluid was not so satisfactory & it Causes the Vacuales to Swell up & the protoplast to enlarge. absolute al cohol causes Vory considerable contraction of the protoplast Distilled water enlarges the Vacuales & causes them to run together by breaking down the Deparating Digitized by Hapt Institutof & Bcytopeab Docionentation the whole protoplast Swells up greatly.

Objection may still be taken to to conclusions drawn from a study of the pallen in the plants own jurie, the Salt Solutions & in 288-while on account aporate operation of leasing out the pollen grains may have exerted.

41 It may still be urged against these abservations that the mechanical operation of paring the anther trasing out the pollen grains man there caused a plasmoly sis of this protoplast That mechanical disturbances the affect the leving contents of these cells is shown by the fact that if the pollen Stains, in 2.8. . 6% Nack Solution, are concred by a cover glass + the pressure due to this is not released & the protopast gradually sularges & may finally Digitized by the unced streating . B & atta pre caution le 101 taken, however, of presenting the pressure of the cover glass by a fragment of andten or filter paper no buch change takes place in the to portage to the best dis objection inter to enimetion of mecrotions from anthers which have been fixed the without subjecting the pollen grains to any such desturbance. Of all the features used the Stronger Flemming's Solution showed the best preservation of the pellen

42 Un error from this cause, however, is extremely improbable as the pollen grains can be drawn out from the anther without a ctually subjecting them to the touch of an instrument + with only very little pressure on priction. This can be done by means of the fibrons mucilese which Surrounds the the pallen grains I binds them together in long strings. Thoreover microtome actions of pollen grains, state in fired whilst lying an untouched within the Jigitizetury, that notivorgar parallel 20 the medanium with those described for presh material. I will not however, great ight on the evidence of the microtone Sections as , in Spile of merg pre cantion, I never succeeded in entirely avoiding Shrinkage of the pallen- protoplast ruen when all the other cells of the anther bere un contracted. I will add here and reasonments of second polen frains from microtone sections comparison between the measurents of the poller for the a loving another with those of monstome "I way the the faith of the fing the start of the forther for the

a comparison between the measurements of the kallen from a living another with those of microtome sections :-43 (1) Fresh material of denothera biennies raammed in the farice squeezed from the anthor :-Pollen grain = "74 fe " cavety = 40 pc " protoplast = 30 pm (2) Sections of another forces of about Same age fixed in Flamming's Solution : -Pollen grain = 72 p. . 70 p. . 70 p. . 70 p. Digitized by Hyptotopostitute for Botanical Documentation

Suring the whole time that the protoplast is separated from the membrane in this way the latter continues to grow both in extent r in theckness. We are at present quite in the dark regarding the manner in which this growth lakes place but a very brief theoretical consideration of the subject will be found among the conclusions at the end of this paper. We must how enquire whence is derived the material hecessary for this growth. Dighine any two is our less to por Bortachic the opensentation material of the membrane might be derived. rig. the protoplast of the pollen grain itself or the tapetum.

That metabolic processes of no mean order are taking place in the former is raident 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 carboky Izates theing used up in the cell; the cyloplasm continually grows less these in arount to whilst a liquid, apparently the derect consequence of the foregoing processes, Digitzed as de unt fostitute for thotapicat pracumentation This lequed first occupies small vacables in the cytoplasm, these continue to increase in (7:924) Size & run together a until we find notting left of the protoplasm but a hollow shell consisting of a plasma (Hautschicht) Ation and lat star this of the centre of the shell is scenfeed by one enormous vacable There is no reason to doubt that this begind (1) Strasburger in his work of 1882 abready ransked wrote of Gaura biennis " In meinen alcaholpräparaten bildet der nach anlage den Wand erschöpfte Inhalt der Pollemgelle nur noch ein unscheinberes Klümpehen" cp. his Fige 48 + 49 Tape VI

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46 diffuses out from the protoplast into the Space which has formed is forming between 116 Tetself + the pollon - wall +, in all probability the latter derives the necessary materials for its growth from this source. Unfortunately I could gain no knowledge whatsoever of the chemical nature of this lequed . In the taketum we can also observe evidences of metabolic activity but I can find ized by Hantshostitue or Bogtanic at Dacative tation is being formed this leaving the cells, on the contrary there is reason to believe that an accumulation of Substance is taking place. On the very young pollen grain the first wall appears as a single homogeneous lamella but when the grain has from & measures about 40 per a cross we can indistinctly recognize a Structural differenentiation in the outer membrane (1) This description of the pollen wall applies bath to Cenath. Congiles of to Den. beennis unless specially stated to the contrary. The measurements more Particlerly refer to O. biennis "but the dimensions are only very stightly definent in the O. Congileona.

4mg When the deameter of the pollen grain has increased still further ( to about 55 to 60 pc) its first membrane can be clearly seen to consist of a then, onter, homogeneous layer + an inner rodlet lager ( stabehenschicht or anschlusslamelle The growth of this membrane recalls Strasburgers description of the forst pallen walk of altheea rosea which, at a certain stage, was deen to consist of three lamellae : a middle rodlet layer (anschlusslamella) which is bounded Digit peripherally by two homogeneous layers . Of these the innermost lamella Soon ceases to grow r becomes gradually more attenuated until it is quote lost sight of altogether; the two other lamellae increase in thickness + the "rodlets" Can be very clearly studied in older Stages a theckening layer is developed within the first pollen walk of alltrace . In Denothera the first wall is so then during (1) Strasburger "Die Aflanzlichen Zellhäute" Pringer. Jahrh. f. Wiss. Bot. B& 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 lemella; it is certain, however, that by the time the pollon grain has reached 55 to 60 per in deameter every trace of it has Vanished. The first pollen walk now grows more rapidly in Durface than the secondary thickening layer beneath it + consequently it becomes Digitz parale unprostitute to Botanie aber gangentation only remains finnly fixed to the interstitial bodies. along The continuation of this unequal growth in Surface goog graduely throws the order walk into erregular of Services folds. Both primary & Scendary layers of the well have meanwhile undergone a change in tues cheme al constitution & take more or less completely caticalaries The secondary layer no longer genes a pure

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Violet reaction with polassium iodide a Solution of Lodine in but this has changed first to a Violet-brown t then to a pure brown 16 reaction . 0.17 The interstitial bodies are shut aff from the cavity of the pollon grain by a well developed closing-dese which consists of two parts. an orter dense & homogeneous layer & an uner less dense, stratified lamina which is cap - shaped in form with the concevity directed Digititor bas flutt Institute for Botaty cal Decret estation lamena of the closing - dese is an aggregation of granular material which extends some little way up the sedes of the interstitual body. Thes cavity of the interstitual body is no longer occupied by the mucilaginous solutions deeply Staining Substance which filed it at an Earlier period, but it now contains a watery fluid which does not readily starn. I believe that the mucclasinons material of the young interstitial body has been to a great satent, used

50 up in forming the closing-dise & that the granular in Substance tokich lies upon the outer portion of the dese is a remnant of the Stamable material. ( see 7:32 23 In pallen grains which measure between i bet 0. biennis + 0. longiflera 85 pe & 95 pe across the protoplast has become reduced to a hollow sphere on Vesicle which the has expanded again until it is nearly on quite in contact with the cell-walk (Fight). At one point upon the Digitipedayellouf Institute for Boplaice Docration total muchanis can be been which encloses a satter large mucheolus. a little finely find Standan cytoplasm surrounds the nucleus but in its other parts the sphere protoplast appears to be reduced to a plasmoderma (Hautschicht) which serrounds le enormons central Vacable. a total Vary finely granular stare Very Soon the nucleus enlarges, & becomes rounder & less dense x passes into the prophases of metotic division (71g 26). «

51 I have not followed the details of this division which leads to the formation of two distinct cells within the pollen grain : the large Vegetatine cell I the small generative cell. The latter is limited (71224) by a well marked plasmodoma ( hauts chicht) The taketum now breaks down I 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 Digitized by Elunt Institute for Beetanic & Doctom detaijon its Earlier development. On the Very young authors, before the fall number of primery sporogenous cells is established, the lapetel cells are felled contain a not very dense cytoplasm which encloses a Single unclears. thes nucleus, besides Small, scattered chromatin granules, contains one to four nucleole. The nuclear membrane colours deeply with oron - hæmatorglin or with methylene blue-fachtin

mixture. Very rapidly the cytoplasm increases in density & the areginally single nucleus divides into deveral, as many as right unclei being not un commonly met with in a cell (Figs 30x31) Until about the pollon - molter - cell Slage the tapetal nuclei multiply the ascelusively by nutotic division but at the matter-cell stage unclear fegures occur which are strongly suggestive of fragmentation. When the special matter-cells have been established mitotic divisions become Digitized by while Inspitation for Botanical Documentation every side. Most of the tapetal muchi how contain a single large nucleolus + a very deeply steering nuclear well, besides this only a very lettle finely granular chromatic material con the demonstrated on the unchange can be seen lying near or upon the unclear well. (see no Fig 44) on anthers in which the first pollen-wall is I have several just making its appearance

53 times Seen the Capital nuclei in the prophases of mitosis but I have never, at this period, Succeeded in finding the later Stages of division + I believe that metodis is no longer completed by the nuclei. Muclei which have every appearance of under going fragmentation are, however, Vory abundant both at this rat later Slages of development (7190 32, 33, 34, 40, 42) Strasbarger + later writers, in describing the tapetum of other plants, have found mitosis to be the Digitized ber Hund denstruit culce Bot addicate Docum datation believe the constructed hucher and occur in the cells to represent fusion r not prequentation of the nuclei . In & acmattera it is impossible to imagine that Rangokinesis can be the only made of miclear multiplecation . It is medicable the the matatic divisions which are none very accordant frequent & are entirely timited to the Steges of devolopment (1) Strasburger E. "Theilungsvorgang & Zelekerne ste" arch. f. Mikro. anat. Bd 21. 1882 pp. 574 - 575.

54 In the first place mitotic devisions are never very frequent & it is deficult to account for the presence of six or seven muchi in the a young tapetal cell through their agency alone. Thoseour, milotic fegures cannot be found in the tapitum of Constains after the appearance of the first pollen wall so that if this is the only made of division + the constructed nuclei which are common both at this I at subsequent stages, really represent Digipized by Hust institute for Bretastical Documentation constant to supply af nuclei comes for there repeated fusions + which leaves the older tapetal cell with two on three nuclee to the last. The way in which these constructed nuclei often hang together by a harrow neck also favours the View that they are deparating from one another + hat uniting (see especially Fig 34) The great disparity in sign of the nuclei you coll an at which can after he watered in the size exists in the Rezes of the nuclei of a all

55 is also shat one would expect with direct Two under my 37 ) rather than with indirect division (compare sizes if the) For all ap these reasons I consider that most of these constructed muclei represent a fraquentation o not a fusion of nuclei Every constricted nucleus does not, however, recessorily imply nuclear multiplication. There is no doubt that the lapetal nuclei alter ther shape + often become very wregular in outle outline without this leading to a Digitized by Hast Lestilute Equis Bognited Doclume station (su Figs 37.36). These changes in shape are evidently signs of the occurrence of an active metabolism in the cell + may he compared to the Semilar phenomena which have been described in the Decreting cells of many animals . This continuous nuclear multiplication, by both derect I enderect deviseon, must lead to the accumulation of a large number of nuclei

56 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 nuclee or cars in the cell + I have most succeeded in finding clear evidence of a unclear degeneration taking place side by Side with the nuclear multiplication. In this process the nuclear membrane, which Slains Very deeply, becomes ruptured + Shredded Digitized byth lunt prohibitation Boston Batanen whilst the nucleolus can also in many instances, he seen to resolve itself into a coarse fibre. There can be little doubt that the groups of fibres formed in this menner correspond to the structures which Meves has recently described in the tapetal cells of hymphaea alba rothich The has compared to the chondromiten of certain animal cells. (1) Meves Fr. "Uber des Vorkommen von Mitschondrien bezw. chondromiten in Pflanzenzellen" Bericht & Deutsch Bot. Gesell. XXII 1904 pp. 284 - 286

54 It is quite easy, in well fixed material, 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 origen, become greenally more numerous in older anthers as the tapetal nuclei continue to devide + to degenerate, but whether they all persect as fibres or and whether Some of them are lost sight of in the Digitized by Hynt posting to Batanica Documentation Say. It is certain, however, that the cytoplasm of the tapetal cells which are approaching disentegration Steens very deeply & that it contains a large number of these fibres. first before tapetal desentegration the whole contents of the cell, apart from the unaltered muclei", break down into coarse granules which Stein intensely with iron-heematorglin + (2) which are two or three in number . (1) Wor cester's fluid is leg far the best fixatine for this purpose.

these become distributed among the pollen grains when the cell loses its individuality. Many of these grandles expected after they be free among the pollon grains appear to undergo a fatty degeneration as they then two they black with some and , either our their entires Surface or more often they makere particles (on trops) which do so. During the development of the anther starch appears + again disappears in the tapetum Digitized by delignt tostillate too duttions cay Dogramentation 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 capelal cells during their development & which Sabsequently passes into the pollen grains to replenech their exhausted protoplasts has

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59 at one time or another entered into the composition of the tapelal nuclei & that there is here, therefore, a direct relation between nuclear Substance of cytoplasmic growth. The comparison which theses has drean between these deeply staining fibres of the tapetal cytoplasm & the chondromiten of certain animal cells is of the highest importance. In a large number of the work that actually functional cells, belonging to the most various Digtizedeby loguntenstitute for Blotagical Documentation Structures have been found in the cytoplasm & described under the names of metochondrien, chondromiten, Abeado chromodomes, yolk-huclei. chromideen, apparato reticolare Etc. Goldschmidt has recently found good grounds for grouping all these structures together the has shown by direct experiment that at least in some cases (2.8. muscle-cells of lescares lumbricoides) they are directly connected with (1) Goldschmidt R. "Der chromidial apparat lebkaft functionirender "Sewebszellen" Zoolog. Jahrb. Abth. J. anst. + Ontogenie d. Thiere B& XXT 1904 J.P. 1-100.

the functional activity of the cell. In the lapetum the fibres (or their derivatives) unquestionably play a prominent part in the cytoplasmic growth of the pollen - protoplasts of no doubt in the anemal cell they are also in some way associated with the elaboration of complex organic substances" In this relation it may be recalled that contain several physiological chemists have pointed out the probability of nuclean or one of its constituent Diginizedeber Hugronptstuferter Boansteath Occustentaryon point for the synthesis of complex organic malters in the leveng cell. The origen & chemical nature of these chromidial Structures thes, however, not yet been satisfactorely determined in all cases. In some cells which have been Studied by Loldschmidt it is hoghly probable that they are dereved from the motion of the nucleus.

(1) For example note the relation which matters found to said hetween the deeply steering fibres + the Zymoten granules of certain poncrees + line cells. Journ. Morphol. SXV Suppl. 1899.

I have shown above that the fibres in the tapetal cells of Denothera possess a nuclear origen + may be referred to the transformation of the nucleoli & nuclear membranes. The Staining reactions + the behaviour of these mucleoli, 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 to Digitized by a cuntoinstitute for totaticate commentation We see therefore that the fibres lying in the tapetal Cytoplasm are to a great estent derived from the chromation of the nucleus + that much of the Dubalance that altimately passes into the pollen greens is a product of chromatin.

mada .

The walls of the tapetum, during the greater part of the development of the anther are of a Somewhat mucclasinons hature & can be very destinctly differentiated by means of an alkaline solution of confo red. In the older anther these walls become Very then I at the time when the lapetum desintegrates they become So alternated that at Some contain Spots they are apparently interrupted altogetter. It is appar obvious therefore that the Digitite place Huets Institute affer Batanjaal Dechindranui or the passage of the cell-contents. It is more difficult however, to understand how the tapetal Substance passes through the thick, cuticalarised pollen-walk to reach the protoplast. It must sudently 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 quessed at. Engymes met probably to formet to has the Effective agents but we at present here

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63 no knowledge sitter og som som e ar the nature of These Satelances . We left the pollen grain at a stage when the proloplast, in the form of a hollow shell, had again enterged sufficiently to fill the cell-cevety once again. Ut a slightly later period the generative cell + the Vegelative uncless leave their peripheral position for one in the centre of the cell Cavity where they are suspended Bigitization with more or less cytaplasin by three Digitization by Houst instigute for Begtoplash Docupentation Smaller ones as well. The three thick arms of protoplasm extend to the bases of the three interstitual bodies + it is a significant fact that the intime can first be recognized at these Spats to that it here altains its greatest thickness. A It is different to avoid the conclusion that influences of Some kind originate in the nucleus + pass to the along the three arms of cytoplasm to those spats where the person advance is depicting landland and forming but in

at which 64 the peripheral pretoplasm where new cell-wall lamellae are forming but we are at present quete in the dark as to the nature of these enfluences . In Still older pollen grains, measuring from 108 to 112 per in deameter, the entire trans completed its the whole einer face of the some secondary theckening lager of the exine. It thick & Easily been at the base of Each interstitial body but it is artremely delicate alsowhere & can only braud as a continuous membrane be demostrated with some difficulty. fighted its Launteln stated for Betanical Documentation yellowet globales which usually entengle (or enclose) an air bulble within them . These global are apparently of an party nature for they are blackened by osmic goed + are soluble in alcohal The intime gives very clearly the characteristic was reactions of a pectic substance but I was and not able to demonstrate the presence of cellabore in it with any certainty. With the Jodene reagents ( Calcium chloride todice or chlor 2 - takine)

65 The interstitial bodies contain one or more yellowish globales which usually entangle an air bubble in them . These globales appear to be af an oily nature for they are blackened by osmic acid & they are Soluble in absolute alcohol. The protoplasm covered by the entire now bores its way through the closing dise & enters the interstitical body which it soon fills. completely. I have followed this process Digitized by House histitut afor Bataniag Daugun Lintekeine a I will therefore refer to this plant in the present description. In the quite young pollen grain of Gaure the interstitute bodies are composed of a Komsgeneous mucelage which in every way redentles that of Denothera at a corresponding age. The older grains this Structureless mucclage becomes distinctly leminated. These laminae are choust Very closely arranged at the base of the interstitud A form there a closing dise . (1) So closely are the laminar arranged into closing dise that the laminated appearance is after cost sight of altogether + the dise appears granular.

above the closing disc the laminae are much more loosely placed & often become to drawn out I even broken at their meddle by the growth espension of the interstitial body. At the spea of the interstitual body the laminae again are very densely arranged. The intime forms quite a thick pad under Each interstitial body but is very then over the rest of the pollen grain . It goes contains both cellulose & a pectic body in its Digitzed by thint Inst Butch of Bottemical and ude the high Equally through the thickness of the membrane " there is no differentiation of a pectre layer from a cellulose one. are about to When the entire & protoplast penetrate the interstitual body we first find that a narrow cleft is & bored through the middle of the closing dise 1. Then a second Small fold of intine can be seen to pass into this Slith +1 gradually make its way to the centre of the interstitual body where the lanunal are thin

64 a small, thick - walled sach The laminae of the interstatual body are gradually Eaten away + the intime - sac continues to grow until it les closely against the short teeth which alone remain of the interstitial laminae. It is interesting to note that the intere must be of a very soft & Suen nuclesenons hature as it moulds itself to all the erregularities on the well + flows between the teeth which project Digitized to Hunter state to the Botanical Defauragentation in the closing disc gradually unlarges until the dese is reduced to a narrow + dense collar or ring. (Fig 50) The manner in which the closing desc is perforated + the substance of the interstitual body slowly eaten away Suggests the presence of a soluent, probably an engryme, which is secreted by the protoplast + which carries out the work of disintegration. It is difficult otherwese to

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

The mature pollen grain of Denothera Confiftoren measures between 140 + 180 pe across; it is Digitized by Hubt Isetitute for Botanical Decementation protoplast which is densely crowded with starch. The two layers of the walk are again in contact with one another. The rodlets or better of the Total layor are nor very conspicious, The outer layer is, however, only firmly allached over the interstitual bodies; it consists of a an onter , tool homogeneous lamella which is continuon our the whole pollen grain & an uner "rodlet" layer which is interrupted over the apices of the interstitual bodies .

Suring the later growth of the pollen grain the Secondary theckning layer has not increased in thickness but has, on the contrary, become Stretched & a good deal thinner them it was at an Earlier Stage. (In Fig 50 the encire secondary thickening layer has been drawn too thick) In Denothere longiflora all the poleen grains do not reach maturity but a large proportion of them become arrested in their development. They mostly grow to about 90p across in Digitized ber Huskenstiteter i procaperson a has been entation reduced to a hollow shell, but there of after that many of them are unable to continue then their development owing, no doubt, to the tapetal substance being insufficient for the requirements of & all the pollen grains. I have not given any rattention to the germenetion of the pollen grain but I may mention that the intine of Epilobium tetragonum

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which genes the reactions of both cellalore & pectore, grows out into a tube which is often branched at its free end ( 41920) The mature pollen grains of Denothera are bound together in long Strings by bundles of "fibrils" which lie between & round them . These fibrils are developed from the muculage which, on an carlier page, we saw was derived from the disintegration of the Special - mother - cell walks. These fibrils have entirely lost all affinity Digitized ballose ages to have be come very resistant to Solaents. Their properties in many respects resemble those of entrealarised Structures on the Species of Epilobium Short bands of the Cuticalarised mucelage binds together the pollen grains an tetrade which are laborated in tetrades consequently leave the anther in tetrads.

Summary + conclusions

(1) In the carliest Stages of anther development all the cell-membranes contain both cellulose + pectore. The wells of the Sporogenous cells, however, contain less cellulore than the other membranes of the anther. In older anthers the Sporogenous cell--membranes give the reactions of a pectic Substance alone. (2) The molter-cell well consists of pure cellose. This substance is formed directly as such by the protoplest + there can be no possibility, in the present case, of a transformation of cellulore into callose.

(3) & the first & second divisions of the pollen-matter cell Digitized laterbloss between for blost in the Doucetie Alisticity fourteen is the approximate number of chronosomes. The presence of two nuclei, one large r the atter very Small, in some of the young pollen grains suggests the occurrence of vieqularities in the divisions similar to those described by Jul in Hemerocellis fulva.
(4) The first pollen membrane is formed by the direct activity of the protoplest + is deposited as a delicate lager of pectic material upon the inner face of the Speciel - mother - cell wall. Although it originates in the second to regime on the second of the second

most intimate contact with the callose walk it is Chemically distinct from this from the Very first. (5) The interstatual bodies originate as specialized spats on the first pallen walk. These spats are at forst characterised by their greater chinness; later a homogeneous muchage is developed at these parts. In old older pollen grains a portion of this muchage is deposited as a dense closing desc whilst, in Denstura, the rest of the interstitual body is filled with a thin fluid. zond by Hunt Institute tor Botanical bacumentation deposited throughout the interstitual body. (6) & Secondary thickening layer is laid down by the protoplast within the first pallen membrane This layer gives most of the pectre reactions but also a very distinct Vholet colour with a Strong solution of toome in polasseism Geodide. It gives none of the usual cellulore reactions. however (7) Both the first pollen well & the Secondary the chaning layer are firming allached to the

Protoplast when they are first developed. In pollen grains which have reached 40 per in Size the protoplast is no longer fixed to the wall at any place although it still completely fills the cell cavety The pollen grain continues to grow to its walls increase both thickness + the surface Whilst the pollen grain doubles its deemster the cell - centy increases in size from about 26/ to about 46 p. The protoplast, however, Digitzed by farme institute tordantagical Demuncates on deameter only enlarges from 26 pe to about 34 pe During the period of the greatest growth of the cell-welt both in theckness & in satent, the protoplast is completely separated promit rether the cellstall can from by intersusception without the direct influence of the living Element of the cell or that element can savet its influence a cross a space fille with fluid. The letter assumption is, highly uprovide a The consecution of the sequelity of some participation of the

In consequences of this inequality in growth the protoplast becomes repareted from the pollen wall by a space which is filled with liquid. The conditions seem to be quite similar to three which Fitting & others have described in the megaspores of Isoetes & Sel Selaginella. In denothera also, as in the megaspores, the growth of the layers of the walk is not Equally rapid & the first pollen walk becomes reparated from the secondary thickening layer + is thrown Digitizated brokentapstitutes four patanical Documentation

These observations show that during the period of most active growth of the membrane ( both in Surface + in theckness) the protoplast is completely separated from it I we must conclude rether that the growth of a cell-wall is a parely physical process or that the leving protoplast can exert its influence across a Space filled with lequid. I may add here that although the growth of a membrane whilst this is separated from the Digitpredetent bystatuita tat deotspic a Draumentation found to occur in the few isolated cases . mentioned above other less satreme instances of the belonging to the same category of phenomena are not unknown. Whereas we find that a new lemelle is interpolated between the protoplast + an older landla + the latter still continues to grow in thickness or in surface it class so whilst it is neither in union or in contact with the leving Slement of the cell.

Theanwhile changes are taking place in the protoplast I 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 ren together until the proloplast is reduced to a hollow sphere enclosing a single, large, central vacuale. We have reason to believe that this fluid diffuses out from the protoplast & furnishes material for the growth Digitized the Hunt Institute for Botanical Documentation The three most important features in the formation I development of the layers of the pollen walk may therefore, he Summarised as follows : -(1) Both the primary pollen well & the Secondary theckening layer oregenate in intimate connection with the plasmoderma (thautschickt). ( The greater portion of the growth of bath these membranes takes place by intuscusception whilst they are completely separated from the protopleat.

(In) The material required for the growth of the membranes is derived from the decretory activity of the protoglast.

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 unbrown's work upon colocales the optical properties of the entrulerised walls, which indicate that the cell-well may be Digitizeder Vaide Institute State ans of the up et a'sion possible that when the membrane is first formed the protoplast ( to which the it is firmly fixed) determines the character & the arrangement of these crystals. to long as the material necessary for the Stout reaches it Each member of this Cry stalling System continues to add her

(1) ambronn H. Ber. d. Deutsch. bot. Gesel 1888 p. 226.

the growth of any other crystal.

The later growth of the membrane, such after it has become separetic from the living Element of the cell, takes place in a manner which indetermined depends upon the nature + relative positions of its crystelline components. (8) after the pollen - prolopeast has become almost completely exhausted by its secretary activity its substance is once more replenished by material derived from the discretegration of the Digitized a setunt Institute for Botanical Documentation The very going lapetern contains a rather Scenty cytoplasm & only a fingle nucleus. at the time when the pallent matter-cells have become Established the tapetal cells are furnished by with denses cytoplasm & their miclai are divident both by metosis + by amitoris. In authors which are and older are lapetal muchei divide les frequently = exclusively by amitodep.

almost (8) after the pollen protoplast has become completely eschausted by its secretary activity its substance is once more replenished by the material derived from the disintegration of the tapetum. The very young tapetum contains a rather scenty cytaplasm + only a sengle nucleus. Later the lapetal cells are furnished with a denser cytoplasm & and made which may vary in number from one to eight. Until the end of the Special - mother - cell stage both Digitizadotte unt lastitute cordectadores lego aun catalion take place but in older anthers the lapetal nuclei divide Exclusively by anitosis Jide 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 staening fibres or harrow laminae. These fibres are no doubt identical with

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those observed by Meves in the taketum of hymphaea alba . These fibres have been compared by menes to the chromedial structures found in certain functionally active animal cells & this comparison has a great interest in the light The chromator fibres in the tapetum of anothera 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 pragmes divide (fragment) Digitized by bluck Institute for Botanical Documentation The interesting conclusion is, therefore, reached that a large portion of the material which replenestes the exhausted pollen - protoplast has at one time or another entered into the composition of a lapetal nucleus. (9) The entine is a continuous membrane living the entire uner surface of the

81 its steatest descopment becaute the at the bases of the three interstitial bodies whilst over turest of the pollen grain it extends as an exceedingly delicate lager. During the development of the entire three thick strands of cytoplasm. connect the centrally placed nucleus with the spots beneath the interstitual bodies where the membrane is frowing most Vigorously. In denothera the intere gives the reactions of Digitized by Hunthinstitute total Stanical Decementation he detected in it. In Gaure Lindheimeri + Epilobium tetragonum both pectic bodies , cellulose occur in the entire. (10) The perforation of the closing disc of the interstitial body + the disentegration of the laminae of that body been have been most closely followed in the pollen grains of Saura Lindheimeri . There is trada to hatiene the The closing desc is perforated

+ the interstitual lamenae are Eaten away" before the in advance of the growing intime in a manner which suggests the action of a solvent, prohably an suggue. (1) & Denothina longiflore, Even when growing under the most favourable conditions, all the pollen frains do not complete their dealopment, tot many a many of the pollen frains become arrested in their development. They all seem to Digitized by Hunt Institute tother otapicate plast mentation completely schausted 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 t bound togetter by fibrils which are dereved from the Special - matter - cell walk. When the Special - mother - cell wall breaks down it forms at forst a Structurellos mucilage which no longer genes any of the

reactions of callone. Later this mullage becomes drawn out into "fibrils" + these are very resistant to Islaents.

Rudolf Beer

## Digitized by Hunt Institute for Botanical Documentation

84 Explanation of Plates Fig 1 Lateral intercellular passage of anther of O. biennis in which the walls bordering upon the space have become the changed & cuticularised. Unliver at stage of special - mother-cells. Figs 2, 3 × 4 Increasing Stages in the development of stoma upon author of O. longiflora. Figo 5-7 Division of pollen malter-cell Showing y chromesomes in O. longiflora Figo 8+9 Later stage of division of same. Att Digitized by Huert Lasifturg for Botaning al. Do all metation from which Figs 5-9 were drawn was fixed with medium chrom-acetec mesitive. Figs 10 + 11. Special - mother - cells Soon after completion of devision. Denothera biennis 71812 + 13. Special-mother-cells later Stage to Show differentiation of the first-formed septa from the later - formed layers of the walk 713/2 Exemened in Color-Zine - codine Solution to which a lette phosphoric - coorine Solution had been added. 71313. Examined in corallin Soda Solution × 600. O. biennis.

7814 Raphide - Sac of anther of O. beennis . Strong Flemming's Sol + Heidenheins heemetoxylen . Figo 15+16 Joung pallen grain of O. beennes immediatly after formation of first pollen--well × 860 Strong Flemming Sol. 7814 You Slightly older pollen-grain of O. biennis . Interstitiel bodies formed × 860. Fig 18. Similar pollen - grain to that in Fig 14 Digitized by Hubit Institute two Batanical Documentation Fig 19 Pollen grein of O. beennis measuring 40 pe in deameter . Living material exemined in . 6%. Nace Solution 7is 20 Branched end of pollen. tube of Epilobium tetragonum 7igs 21+22 abnormal pollen greens of O. biennis with two + one interstitual body respectively . Transmed in . 6% Nace Solation 78 23 Pollen grain of O. biennis 45 le across Hernming's Solution . meltiglene blue & fucksin

Pollen grain of O. longiflora 70 pe across 718 24 Living material examined in . 75% Nace Solution Rollingrain Section of pollen grain of 71825 aborgiflara O. biennis meeturing about 86 pe across the entire grain to a when it has again expanded inte it nearly fills the cell - centry of secretory activity & Hermings Sal. Heidenhains haemeloxylin & beswarek brows Dizigized by Hendeartitysteelear gratianized the constantion nucleus showing forst stage of metoris 71827 Lection of pollen grain of O. beennis reasuring about 86 p across showing Vegetatine + generative (g) cells. Flernming's Sol. Heidenhain's Reematorglin + bismarck brown. 718 28 Pollen grain of O. beennis measuring 150 pe a cross to show the three arms of protoplasm estending to the bases of the three interstitual bodies where the

83 intere was just beginning to be formed Leving material examined in . 6% Nace Solution Fig 29 Pollen grain O. longiflora to show the three arms of cytoplatin. Fixed with Worcesters fluid . Methylene blue & fuchsin stein × 860 Tapetal cell of O. longiflora with 48 30 8 nuclei . Strong Flemming 206. meltiglene blue + fucksin preceded by croceen steen. Dizitized by Mapletasticele for Botenigatoracuite statice Figs 32-34 Tapetal cells of O. biennis to show derect devision of nucleus. Strong Flemmings Solution Tapetal cells to show constructed Fig 35 - 39 + orregular uncles some of which and be stages of direct division to others no such de grificance attachés. 7: 80 40 - 42 apetal cells from Domewhat older anthers in which pollen grains measure from 60 to 95 pe across. To show constricted nuclei.

718543-45 Tapetal cells of O. longiflora to show chromidial fibres (f), degenerating nuclei (d) & intact nuclei (n) . Worcesters fluid; methylene blue & fuchsin × 860 Fig 46-49 Interstitical bodies of pollen grain of Gaura Lindheimeri showing Successive Stages in its perforation & dessolution by the advancing intime Figs 46-48 examined in chlor-Time-Digitized by Hunt Institute bela tratanized & qculteentetion with bismarck brown. All feared with Flemmings Solution . 71850 Interstitial body of mature pollen green of O. longiflora. medrum chrom - acetic . Bismarck brown x 860. Fig 51+52 Disintegration of special - mother-- cell- well . Den . beennis Strong Flemming . To show the first-formed lamellae of the well still left intact whilst to later - formed layers have completely broken down . Fig 51 congo red stain . Fig 52 corallin sode .