Notes on the Development and Structure of the Seed in the Alsinoideae.

BY

L. S. GIBBS, F.L.S.

With Plates V and VI and four Figures in the Text.

Introduction.

THE Caryophyllaceae, from the point of view of embryological development, seemed to have been rather overlooked in recent years, in comparison with the large amount of work which has been done in this particular line of research for other families and individual species.

This was not the case with the older botanists. Schleiden and Vogel (2), Meyen (3), Tulasne (5), and Hofmeister (6) all record interesting conclusions on the morphological development of the suspensor, embryo and embryo-sac. But most of this work, good as it is, is incidental or comparative, and there is no consecutive account of the embryology and development in any one species.

In this order the abundance and persistence of the nucellar perisperm is a marked characteristic, and the formation of this tissue has been followed with interest by several authors. Schleiden and Vogel (2) correctly figure the peculiar shape of the embryo-sac and the localization of the starch storage tissue, describing the former as growing in horseshoe shape round the main mass of the nucellus (perisperm) of which it destroys only the peripheral layers. Hegelmaier (10) defines the limits of the permanent nucellar or perisperm tissue in this order, as the incidental result of working on the morphology of the endosperm in both the groups Silenoideae and Alsinoideae which constitute it. He does not suggest any possible physiological relation between these tissues, and describes endosperm formation in these ovules as transitory in character.

Recently Johnson (23, 25, 26) has worked out very thoroughly the embryology and germination of certain Piperaceae, and one of the chief results of his investigations is to bring out the important rôle played by the endosperm in the development of the embryo.

He draws some interesting conclusions from this fact on the function of the endosperm in all seeds containing abundant perisperm. In *Peperomia pellucida* and in *Heckeria* the endosperm is described as bursting out of the

[Annals of Botany, Vol. XXI. No. LXXXI. January, 1907.]

seed-coat and continuing to jacket the embryo, which at germination is only an undifferentiated mass of cells, until the root, hypocotyl, and cotyledons are organized. This endosperm is not a storage tissue, but digests the perisperm reserves and passes them on to the embryo. The suggestion is therefore made that this restriction of the function of the endosperm obtains in all seeds with abundant perisperm, the sporophyte of the second generation being nourished, not by the parent generation, but by the intervening gametophyte.

A chance series of sections through a mature seed of *Stellaria aquatica* which seemed to prove the justice of this point of view, led to the present investigation which rather confirms Johnson's hypothesis.

To understand the organization of the mature seed, it was necessary to trace the separate tissues composing it to their origin, and the subsequent results seemed sufficiently interesting to justify publication.

Great uniformity and simplicity of structure prevails in all members of the Alsinoideae examined.

This fact renders the progressive and comparative development of the nucellar tissues, in conjunction with that of the embryo-sac and embryo, easy to follow. Some stress has been laid on this point, as the part played by the separate tissues of the ovule in the development of the embryo are especially accentuated in this case, owing to the early laying down and abundance of the perisperm coupled with the restriction of the functions of the suspensor and endosperm.

Morphologically these ovules are characterized by the constant presence of two integuments, each composed of two layers of cells, the inner integument always projecting beyond the outer one. The nucellus increases in size by the periclinal and anticlinal divisions of the epidermal layer, and this results in the sinking of the embryo-sac in the nucellar tissue, and the formation of a sort of transitory beak at the apex of the nucellus by the outgrowth, prior to fertilization, of certain cells which are subsequently reabsorbed.

The chief feature in the embryology is the filamentous suspensor, the basal cell of which (that directed towards the micropyle) attains to a very large size.

The uniformity that characterizes the endosperm in these ovules is very striking, and one of the chief objects of this research is to determine the function of the cells of this tissue in relation both to their morphological differentiation, and to the nutrition of the embryo.

Variation being so slight in the tribe Alsinoideae, one species is taken as a type.

Stellaria media, L., was chosen as offering a good example, and it was studied as far as the maturation of the seed, partly because the basal suspensor cell reaches its maximum development in this species.

In the stages of early development investigation is difficult owing to the peculiar orientation of the ovules and the position of the loculi. Transverse sections are useless and good longitudinal ones a question of luck. For drawings of these stages, therefore, where better preparations were available in other species they have been substituted for *Stellaria media*. As the ovules mature, gradual changes take place in the seed coat, which result in cuticularization of the cell-walls and infiltration of tannin into both the cell-walls and contents of the tegumentary layers. The tannin is very resistant to the penetration of paraffin and good microtome series are difficult to obtain. For the study of the germination of the seed, *Cerastium perfoliatum* was found to offer a favourable example, as it germinates easily, and the walls of the seed coat are not so cuticularized and contain less tannin than in most other species.

Three distinct stages seem to mark the comparative development of these ovules, viz. :—

1. Pre-fertilization.
2. Post-fertilization to maturation.
3. Germination of the seed.

The descriptive matter has been arranged accordingly.

In this course of development two long rests occur :—

(*a*) in the pre-fertilization stage, immediately after the fusion of the two polar nuclei into one definitive nucleus ;

(*b*) on the maturation of the seed.

In explanation of the terms employed, *primary megaspore* stands for the megaspore mother-cell, which develops directly into the embryo-sac, since no subsequent tangential divisions of the primary megaspore cell were observed. The development of the embryo-sac is thus similar to that obtained in many lilies. *Primary suspensor* refers to the whole of the filamentous row of cells preceding the actual embryological divisions, which in the early stages is usually called the pro-embryo.

As the inner and outer integuments are each composed of two layers of cells, these layers are referred to as layer 1 and 2 of the inner integument and layer 1 and 2 of the outer integument respectively, starting from the periphery of the ovule. The functions of these two layers, being dissimilar in the case of the outer integument and similar in that of the inner, it is obviously necessary to differentiate between them.

The lower portion comprises the base of the nucellus and the chalaza.

The species investigated were as follows :—

A. As far as the definitive nucleus stage.

1. Alsineae.

Stellaria Holostea, L., *S. media*, Cyr., *S. graminea*, L., *S. uliginosa*, Murr., *Cerastium glomeratum*, Thuill., *C. quaternellum*, Fenzl,

C. perfoliatum, L., *Sagina apetala*, L., *S. procumbens*, L., *Moehringia*, sp.

2. Sperguleae.

Spergula arvensis, L., and *id.* var. *sativa* (Boenn), *Spergularia rubra*, Pers.

B. From the definitive nucleus stage as far as the maturation of the seed.

1. Alsineae.

Stellaria Holostea, L., *S. media*, Cyr., *S. aquatica*, Scop., *Cerastium glomeratum*, Thuill., *C. perfoliatum*, L., *Sagina apetala*, L., *Alsine trinervia*, L.

2. Sperguleae.

Spergula arvensis, L.

C. Germination of the seed.

1. Alsineae.

Stellaria Holostea, L., *S. aquatica*, Scop., *Cerastium perfoliatum*, L., *Alsine laricifolia*, Wahlenb., *A. fasciculata*, M. Koch.

2. Sperguleae.

Spergula arvensis, L., and *id.* var. *sativa* (Boenn), *Spergularia salina*, Presl.

Historical.

The results of former work on the group are as follows :—

Grew (1) in 1682 figures the seeds of *Spergula* and chickweed, describing the former as 'spherick in shape with a knobbed surface and membranous Rimm,' and the latter as kidney-shaped.

Schleiden and Vogel (2) in 1839, in a paper on 'Albumen' first distinguished between 'perispermium' or storage tissue derived from the nucellus and 'endospermium' or tissue derived from the embryo-sac. They give a very good figure of *Spergula pentandra* with the small celled suspensor which characterizes the Sperguleae, and in a series show starch storage tissue limited to the central mass of the nucellus, which alone persists as the embryo matures.

Meyen (3), 1841, working on *Stellaria media*, noticed the elongation of the suspensor beyond the embryo-sac peculiar to this species. He figures the characteristic twist of the pollen-tube, where it adheres to the apex of the embryo-sac. Following Schleiden, he interprets it as the beginning of the 'vésicule embryonnaire' which he describes as developing first into the apical portion of the suspensor and ultimately into the suspensor and embryo. He considered, that from its size, the suspensor must absorb food material for the embryo.

Unger (4), 1855, figures the style of *Stellaria media*, with a pollen-

grain on a papilla of the stigma, through the wall of which it is supposed to penetrate.

Tulasne (5), 1855, in a most beautiful series of drawings from dissections of the embryo-sac, with embryos in different stages, of *Cerastium triviale* and *C. collinum*, *Holosteum umbellatum* and *Stellaria media*, dwells on the peculiar form of suspensor (vésicule embryonnaire) in the latter, calling the prolongation the 'appendice.' He figures the twist of the pollen-tube at the apex of the embryo-sac, and the persistence of the same long after fertilization as general for species investigated, which differ chiefly in relative size and shape of suspensor.

He describes the suspensor in *Spergula arvensis* and *Spergularia rubra* as much simpler and almost uniform in diameter.

Hofmeister (6), 1858, in *Stellaria media* saw the two synergideae and oosphere (Keimbläschen), but no antipodals, and spoke of the upper ends of the 'Keimbläschen' as being pressed against the 'spitze Ausstülpung, welche die flache Scheitelwölbung des Embryosackes in ihrer Mitte trägt.' He noticed the persistence of the synergideae (unfertilized 'Keimbläschen') till the first division of the fertilized one, when they are quite 'verdrängt' so that only the upper portion of the first cell of the pro-embryo (Keimbläschen) occupies the 'Ausstülpung' of the embryo-sac.

He described the suspensor (Embryoträger) as long in all Caryophyllaceae and the endosperm as scanty and as appearing late.

Vesque (9), 1878, working on the development of the embryo-sac in Angiosperms, found that in *Stellaria Holostea*, the primary mother-cell was hypodermal in origin, developing directly into the embryo-sac without further tangential divisions. He also figures the development of a 'nucellar cap' by increased periclinal divisions.

Guignard (18), 1882, in *Silene obtusifolia* saw two tangential peripheral divisions in the mother-cell, but admits not being able to trace real succession, owing to the slight differentiation between them and the rapid enlargement of inferior cell into the embryo-sac.

Godfrin (15), 1880, working on the seed coats of Angiosperms found such marked uniformity of structure in the Caryophyllaceae (Sileneae and Alsineae) as to be characteristic of the tribe. He figures the seed coat of *Spergula arvensis* in transverse section.

Hegelmaier (11), 1885, in his paper on the Morphology of the Endosperm of Dicotyledons, places the Caryophyllaceae in his third class of 'einseitig peripherischen,' in which the endosperm first lays down one layer at the micropylar end, then develops centripetally, filling up the apical portion of the embryo-sac.

Working on *Stellaria Holostea* for the Alsinoideae, he denies free cell formation at the chalazal end, where the endosperm nuclei merely degenerate, and describes the apical tissue as lasting only for a short period.

The same author in another paper (12), 1895, on the 'Orientation of Embryos in Dicotyledons,' shows that the curvature of the embryo in the Curvembryae is due, in the first instance to no mechanical pressure, as enclosed for a long period in transitory endosperm, it touches neither perisperm nor testa. He mentions the small celled suspensor of *Spergula arvensis*, the spiral position of the cotyledons in the mature seed for that species and their thick and narrow consistency.

Holfert (20), 1890, on the proteid layer (Nährschicht) in *Stellaria nemorum*, mentions three layers as composing the testa of the seed, viz. (1) an epidermis, with wavy cuticle and contracted protoplasm in places; (2) a 'Pigmentschicht' of tangentially stretched cells with brown contents; and (3) a layer of cells bulging towards the inside, parenchymatous and without contents, consequently 'Nährschicht,' as the contents must have been absorbed.

But in *Spergula arvensis* he gives the sequence of the three layers, as (1) epidermis (growing out at intervals into club-shaped hairs), (2) 'Nährschicht,' brown and obliterated, and (3) colourless quadrate cells (in transverse sections) with pitted walls and brown contents.

He worked on mature seeds only.

Balfour (27), 1901, in his comprehensive address on the Angiosperms, throws out some illuminating suggestions as to the function of the integuments as an integral portion of the sporangium, apart from their ultimate purely protective use in the ripe seed.

He describes the tegumentary system of the ovule as an outgrowth of the sporangial primordium of variable origin and development, its primary function in Angiosperms is regarded as being that of water jacket and food store, developed in response to special demands for water involved in the seed habit.

Finally, Johnson (26), 1902, in the germination of the seeds of certain Piperaceae describes the formation of the endosperm, and calls attention to the fact that it is not a storage region, but digests and passes on food material to the embryo from the more abundant perisperm or storage tissue, and he suggests that this same relation between perisperm and endosperm obtains in all seeds with abundant perisperm, such as the Polygonaceae, Chenopodiaceae, Phytolaccaceae, and Caryophyllaceae.

Comparative Development of the Nucellus and Embryo-sac, as far as Fertilization.

Stellaria media.

To study the growth of the nucellus, the very earliest stages in the development of the flower must be examined. After the laying down

of the carpels on the flower rudiment, a conical portion forming the extreme apex of the latter remains, and it is this axile apical portion which grows on in the centre of the carpels, forming the columella. The growth of the columella is at first more rapid than that of the carpellary whorl. The ovules arise on it in basipetal succession, and the ovular outgrowths appear on the top of the columella before it is enclosed by the carpels. The nucellus first consists of a one-layered epidermis and some hypodermal cells. As it increases in length by anticlinal divisions of these cells a larger hypodermal cell is soon distinguishable (Pl. V, Fig. 2, *m.*) terminating the axile row of the nucellus. This cell is the primary megaspore, and as Vesque found for *Stellaria Holostea*, it becomes the functional megaspore without further tangential divisions. Anticlinal divisions now appear in some of the epidermal cells of the nucellus, which if occurring over the megaspore, may simulate tangential divisions of the latter (Pl. V, Figs. 3 and 5). In *Stellaria uliginosa* in two cases, exceptions to this rule were seen (Figs. 1 and 2, *t.* and *m.*) ; but in Fig. 2 the apparent tapetum may be derived from the epidermal layer, the section being possibly oblique.

As the primary megaspore enlarges, two or three of the cells below it in the same vertical row become differentiated from the surrounding tissue in size, denser contents, and in larger nuclei (Pl. V, Figs. 3 and 5, *ax. c.*), and it is at the expense of these cells that the subsequent growth in length of the megaspore takes place.

The cells of the nucellus in immediate contact with the megaspore also show larger nuclei and denser contents, simulating sporogenous tissue. Some caution is therefore necessary in the interpretation of even slightly oblique sections.

The integuments arise in basipetal succession.

Embryo-sac. The first division of the nucleus of the megaspore occurs before the inner integument encloses the nucellus (Pl. V, Fig. 5, *e. s.*). Subsequent divisions to the eight-nuclei stage follow in normal sequence (Fig. 6, *e. s.*). Very rapid anticlinal, and less rapid periclinal, divisions of the epidermal layer of the nucellus follow (Pl. V, Fig. 6, *per. l.*), with the result that the embryo-sac becomes sunk in its tissue and is enclosed in four or five concentric layers which join on to the axile rows at the base of the nucellus (Figs. 6 and 7, *per. l.* and *ax. c.*).

Increased anticlinal divisions occur at the apex of the nucellus (Fig. 4, *ap. nuc.*), also noted by Vesque (9), who speaks of the 'nucellar cap.' The increased periclinal divisions he figures for the 'cap' were not seen, and each layer in every case can be traced all round the periphery of the nucellus in all stages of the growing ovule. These cells, at the immediate apex of the nucellus just under the micropyle, form loose vertical rows (Figs. 6, 7, *ap. nuc.*), the extreme cells of which, just before fertilization, are prolonged as papillae into the micropyle (Fig. 13, *ap. nuc.*).

The embryo-sac after elongating at the expense of the primary axile row of cells (Figs. 5, 6, 7, *e. s.*), expands by the absorption of the limiting concentric nucellar layer, which in this stage shows starch contents (Figs. 6 and 7, *per. l. st.*). Progressive digestion of these limiting layers is shown by the existence, in contact with the embryo-sac, of disorganized cells, which remain in the position of, and can be traced back to, the layer of which they formed part (Pl. V, Figs. 6 and 7, *dis. c.*).

At this stage the embryo-sac contains eight free nuclei, and these quickly differentiate into the two synergidae and oosphere, three antipodals and two polar nuclei (Fig. 6, *syn. oos. ant. p. n.*). The cells of the nucellus in the immediate vicinity of the antipodals present the same unattached and partially digested appearance as those surrounding the rest of the embryo-sac (Figs. 7 and 8, *ant.*). No evidence points to the antipodals influencing the solution of tissue in contact with them. They are ephemeral, disappearing after fertilization ; and even before their differentiation the disintegrating changes in the nucellus are apparent. This seems to show that the actual cytoplasm of the embryo-sac is the active digestive agent up to fertilization, and also to a certain extent afterwards.

The synergidae are well defined. They are long cells, and contain large nuclei (Fig. 7, *syn.*).

Fusion of polar nuclei. The fusion of the two polar nuclei into one definitive endosperm-nucleus occurs some time before fertilization (Fig. 7, *d. n.*). The definitive nucleus resulting from the fusion is very large, with a well-marked nucleolus and dense reticulum. It occupies the centre of the embryo-sac towards the upper portion, and is in contact with the oosphere. The latter, which lies against the synergidae and near the embryo-sac wall, is smaller with a more alveolate reticulum (Fig. 7, *oos.*).

The fact that the nuclear membranes of the definitive nucleus and oosphere are in contact is characteristic of *Stellaria media* (Fig. 9, *d. n.*). In other species examined, this was never found to be the case, some cytoplasm always intervening (Pl. V, Fig. 8, *d. n.*). At this stage a very long rest occurs. It is on that account the most easy to obtain, and was found to occur in sections both of the expanding bud and open flower.

Progressive development of endosperm, perisperm, and embryo after fertilization. The pollen-grains settle on the papillae of the stigma, as was correctly figured by Unger (4), the tubes growing *along* the cell-walls, but not *penetrating* them as he describes. They continue to force their way between the cell layers composing the tissue of the style which is in direct continuity with that of the septa of the ovary.

These septa consist of loose, spongy tissue which forms papillae on each surface, and it is on these papillae that the micropyles of the ovules abut. This fact explains the definite orientation of the two rows of ovules in each loculus.

Miss Lister (19) notes the spongy nature of the septa in this group, and suggests their probable function as conducting-tissue for the pollen-tubes. In the course of the present investigation, tubes were repeatedly seen in the septa of the ovary, and also penetrating through the papillae which clothe their surface, to the micropyles of the ovules. These papillae persist after fertilization, and, as the septa break down as the ovules increase in size, the ruptured surface on the columella becomes covered by similar outgrowths of the cells. They evidently serve to ensure the nutrition, and form the conducting-tissue for the pollen-tubes in their passage to the ovules. In *Arenaria tenuifolia* these papillae elongate considerably, entirely filling up the cavity of the ovary. They replace to a certain degree the septa, all of which become broken down by the growth of the ovules, and possibly they may serve as paraphyses to keep the ovules damp.

As the pollen-tube makes its way through the conducting-tissues of the style and septum of the ovary to the ovule, some modifications take place in the latter. The contents of the fan-like rows of cells forming the extreme apex of the nucellus, as previously described on p. 31, show increased density and darker staining properties, and the terminal cells of these rows grow out as long papillae into the micropyle (Fig. 13, *ap. nuc.*).

The inner integument projects far beyond the outer, and the cells of which this projecting portion is composed show considerable increase in size and darker staining properties (Fig. 7, *i. i.*). The contents of these cells are used up by the growing pollen-tube, the walls shrink, leaving a cavity, and it is this cavity that the papillose outgrowths of the nucellar apical layers project.

The function of these cells is probably to ensure the nutrition, and to facilitate the passage, of the pollen-tube to the embryo-sac. Their subsequent absorption by the pollen-tube leaves a channel from the apex of the nucellus to the embryo-sac, in which the tube persists for a long time (Pl. V, Figs. 17 and 18, *p. t.*).

Before entering the synergidae the pollen-tube forms a slight swelling, and the apex then forces its way between them and lies against the oosphere (Fig. 9, *p. t.*). Further penetration into the cavity of the embryo-sac was not observed. The pollen-tube in all other species of the Alsinoideae examined is very thick and persistent, forming a very characteristic twist on itself before penetrating the synergidae (Figs. 13, 17, *p. t.*). But *Stellaria media* forms an exception to this rule. Tulasne (5), in his drawings of dissections of the embryo-sac with suspensor and embryo, figures the twist adhering to the apex of the embryo-sac in every other species examined by him, and he lays stress on the fact that it was impossible to dissect out the one without the other. In *Stellaria media*, however, this was not the case, and he found it difficult to isolate an embryo-sac with the pollen-tube still in contact, the latter remaining in the micropylar region and in the apical

portion of the nucellus. In this species the wall is much thinner, and the granular remains of contents are not so apparent; the tubes are therefore more difficult to see except in section, and the usual twist formed on the entrance to the embryo-sac is replaced by a slight swelling of the tube (Fig. 9, *p. t.*).

Endosperm. After fertilization, as the oospore surrounds itself with a cell-wall, the definitive nucleus elongates and prepares to divide (Fig. 9, *d. n.*). This division, which never takes place before the first segmentation of the fertilized egg, is extremely rapid; amongst all the material examined not a single case of actual primary division was seen.

In preparations, however, showing the first division of the oospore, seven or eight endosperm-nuclei have been counted in a series of sections through the embryo-sac, and this was found to be the average number for this stage. These nuclei migrate at once to the periphery of the embryo-sac, where they lie embedded in the cytoplasm, merely dividing to keep pace with growth. At the micropylar and chalazal ends of the embryo-sac more rapid divisions occur, leading to aggregations of nuclei and a denser mass of cytoplasm at each extremity of the sac (Fig. 14, *end. c.*; Fig. 16, *e. s.*).

It has been shown (p. 32) that up to fertilization (Figs. 6 and 7) the uniform solution of the layers of nucellar tissue immediately in contact with the periphery of the embryo-sac points to its cytoplasm as being the digestive agency. This digestion after fertilization receives a definite impetus by the aggregation of endosperm-nuclei at the *antipodal* end of the sac (Fig. 16, *e. s.*), which elongates rapidly at the expense of the axile rows of the nucellar tissue situated immediately below the antipodals (Figs. 18, 19, *ax. c.*) until the wall of the embryo-sac arrives in proximity to the chalaza, where it enlarges somewhat (Fig. 19, *e. s.*).

A comparison of Fig. 7, in which the embryo-sac is fairly vertical and is exercising a destructive influence on the cells over its entire periphery, with Fig. 19, shows that some definite stimulus must have caused so distinct a line to be taken by one particular portion of an organ, and this fact is to be correlated with the aggregation of endosperm-nuclei at the chalazal end of the embryo-sac.

As will be shown later, there is reason to suppose that certain layers of cells at the base of the nucellus are specialized in a form which suggests a tissue for the passage of air and water.

Endosperm cap. Very active divisions of the endosperm-nuclei at the micropylar end of the embryo-sac result in an aggregation of nuclei, embedded in dense cytoplasm, in the vicinity of the basal suspensor cell, thus forming a cap surrounding it (Fig. 14, *end. c.*).

At about the time at which the cotyledons are first differentiated these endosperm-nuclei arrange themselves peripherally (Fig. 19, *end. c.*), free

cell formation subsequently follows, resulting in the formation of a single layer of small quadrate cells with dense protoplasmic contents (Figs. 20, 21, *end. c.*). This layer of cells keeps pace with growth by means of constant anticlinal divisions at the apex of the embryo-sac, but it gradually thins out into large uninucleated cells with vacuolated contents over the remaining portion (Fig. 21, *end.*). In the mature seed the endosperm-cap invests the root-cap of the embryo, and the rest of the embryo-sac is lined by a thin film of very large cells. The embryo is therefore enclosed by a single continuous peripheral layer of endosperm-cells of diverse character in the hypocotyledonary and cotyledonary regions respectively. In sections the cells composing the larger portion of the layer are difficult to see, owing to their size and extreme thinness, but they are easily differentiated by careful staining, and this portion can be dissected out in its entirety; but it is very difficult to get any part of the cap off on account of its intimate relation with the root-cap of the embryo on the one side and the nucellus on the other.

In consideration of these facts Hegelmaier (12) is hardly correct in describing the embryo as being enclosed in transitory endosperm in the Curvembryae, a group in which he had previously stated the endosperm to be limited entirely to the micropylar end (10).

Cell-wall formation in the endosperm is recorded for several families at a stage similar to that at which it occurs in *Stellaria media* of the Alsinoideae, that is, when the cotyledons first become differentiated. Guignard (17) records it for the endosperm of some Leguminosae, and concludes it becomes definitely organized at this stage to meet the increasing requirements of the embryo, the suspensor, it is assumed, being now no longer capable of doing so.

Strasburger (30), for the Eualchemillas, states that the endosperm forms walls as the embryo becomes heart-shaped. He makes the apposite suggestion that, as the embryo-sac is then full-sized, the stoppage of growth causes the endosperm-nuclei to remain in contact and so start cell division (30, p. 124). That this observation applies in the present case also is borne out by the regular arrangement of the nuclei of the endosperm-cap (Fig. 19, *end. c.*), which obtains just before cell division takes place. Péchoutre also (28) arrives at a similar result in the case of the Rosaceae.

In *Stellaria media* we see that the endosperm is differentiated in its apical portion into a compact layer of cells with dense and homogeneous contents, which in organization and appearance strongly suggest ferment-cells. This cap invests the apex of the embryo with its inner surface, whilst externally it is in direct contact with the axile rows of the nucellus (Fig. 22, *end. c.*). The cells of these rows adjoining the endosperm-cells show paucity of contents and very slight starch reaction, but they abut directly on the perisperm tissue of the nucellus, the latter appearing as

if it were constantly being drained of contents. There is no peripheral solution of tissue, as is the case in the vicinity of the basal suspensor cell, the endosperm layer merely consisting of passage cells. The entire absence of any other form of secretory tissue can only lead to the conclusion that the endosperm in this case is the medium through which the starch stored in the perisperm is made available for the growing embryo.

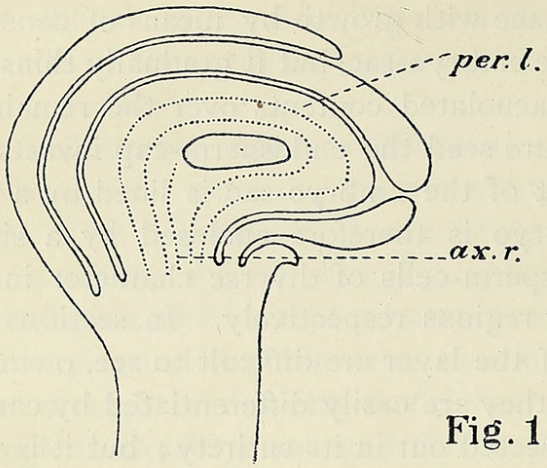


Fig. 1.

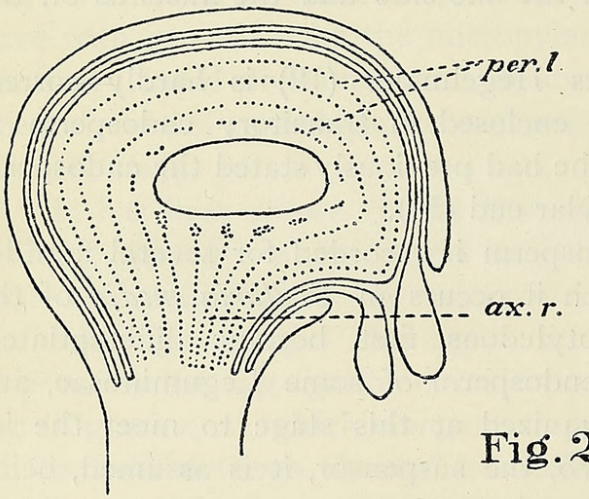


Fig. 2.

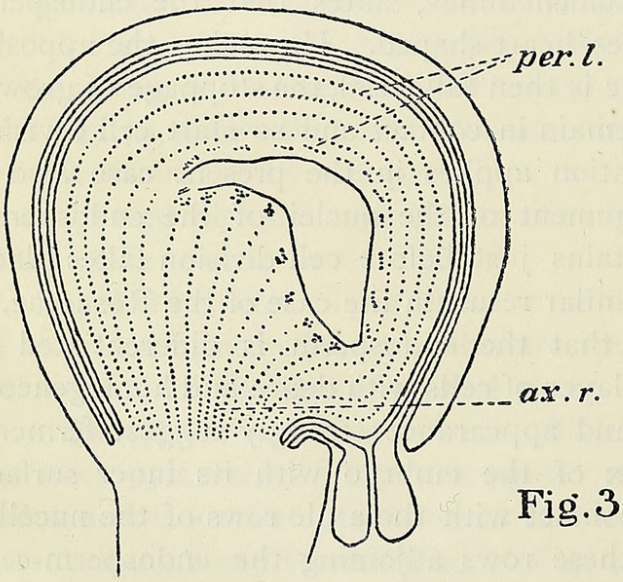


Fig. 3.

Figs. 1–3. The dotted lines represent the cell layers, and when interrupted show digestion of the same.

The Axile Cells of the Nucellus and the Perisperm. Before fertilization, continual vertical growth in the basal region of the nucellus results in an increase of the axile rows (*ax. r.* Text-figs. 1–3). Anticlinal cell division of the peripheral layers (*per. l.* Text-figs. 1–3), which from the earliest stages of development, is less active on one side than the other, now ceases altogether on the lower side, and the embryo-sac is thus forced from a horizontal to a more or less vertical position, by the campylotropous curvature of the nucellus characteristic of the order.

The axile cells are in serial connexion with the chalazal cells, and after fertilization two or three of the basal rows become vacuolated and the nuclei migrate to the walls, which cuticularize (*b. c.* Text-fig. 4, p. 38). These cells form a band across the chalaza connecting up on each side with

layer two of the inner integument (*i. i.* Text-fig. 4), the walls of the latter also becoming cuticularized (Pl. V, Fig. 18, *b. c.*). In a longitudinal section through the chalaza and base of nucellus (Fig. 12, *b. c.*) these cell layers are shown between the small polygonal cells of the chalaza and the rectangular layers of the nucellus. Their continuity with layer two, of the inner integument, can also be traced. The walls of these basal cells show pores, which do not occur on the cuticularized walls of the inner integument. These pores probably allow the free passage of water, for in these ovules the vascular tissue of the funicle does not penetrate beyond the chalaza (Figs. 18, 19, *v. b.*), and there is no vascular system in the nucellus or integuments. Nawaschin (22) figures a cuticularized area of thickened cells in the chalazal region of the elm ovule, which suggest comparison with the basal cells described above for the Alsinoideae. No further explanation is offered in the text.

Godfrin (15) notes that orthotropous and campylotropous ovules vary only in the nucellus being straight or curved. In both cases the hilum is directly under the nucellus and, with few exceptions, the seeds are non-vascular. Though he does not explain the fact, possibly the advantageous position of the chalaza, or hilum in the mature seed, has something to do with the efficient distribution of water, without the supplementary aid of a branching vascular system. The fact that as the ovules mature the terminal vessels of the funicle branch freely in the chalazal tissue may be adduced in support of this view (Figs. 18, 19, *v. b.*). The reserve food material is laid down directly over this the sole source of water supply, the cuticularization of the inner integument after fertilization, as described above, effectively cutting off all other channels.

In the mature seed and on germination large air spaces occur in the angles of the walls of the first two or three layers of the axile nucellar cells immediately above the cuticularized basal cells, which suggest the possibility of these latter cells forming a sort of aerenchyma (Pl. VI, Figs. 22 and 24, *a. s.*).

Perisperm. Before fertilization starch is limited to the layer of nucellar cells adjoining the embryo-sac (Pl. V, Figs. 6, 7, *st.*), the axile cells of the nucellus being as yet undifferentiated in respect of size and contents. After fertilization, however, starch is laid down very actively in those axile cells which immediately abut on the embryo-sac (*prm.* Text-fig. 4), and in this way the process of development of the perisperm is inaugurated in the nucellus (Pl. V, Figs. 16 and 18, *prm.*). Progressive development of the perisperm occurs (Pls. V and VI, Figs. 19, 22, *prm.*) until it forms a tongue of cells so densely packed with starch grains that the nuclei are squeezed out of all shape, but as the base of the nucellus, towards the chalaza, is approached, the starch contents become less and less, the cells are much smaller, the nuclei more and more active and the cytoplasm denser in

consistency, and quite at the base active cell division goes on, till maturity, when all the cells contain starch. This perisperm exercises a mechanical influence on the shape of the embryo-sac. The thin cytoplasm of the same has no effect on the dense contents of these cells (*prm.* Fig. 4). Careful investigation shows no sign of digested cells on the convex portion of the perisperm cells of the nucellus. Increase of breadth being thus effectually prevented on each side by the limits of the perisperm and testa respectively, the necessary expansion of the embryo-sac must take place in length and the natural effect of such a rigid mass of tissue is to increase the convexity of both embryo-sac and embryo. Hegelmaier (12) explains this development as a 'crescent-shaped portion of the nucellus which

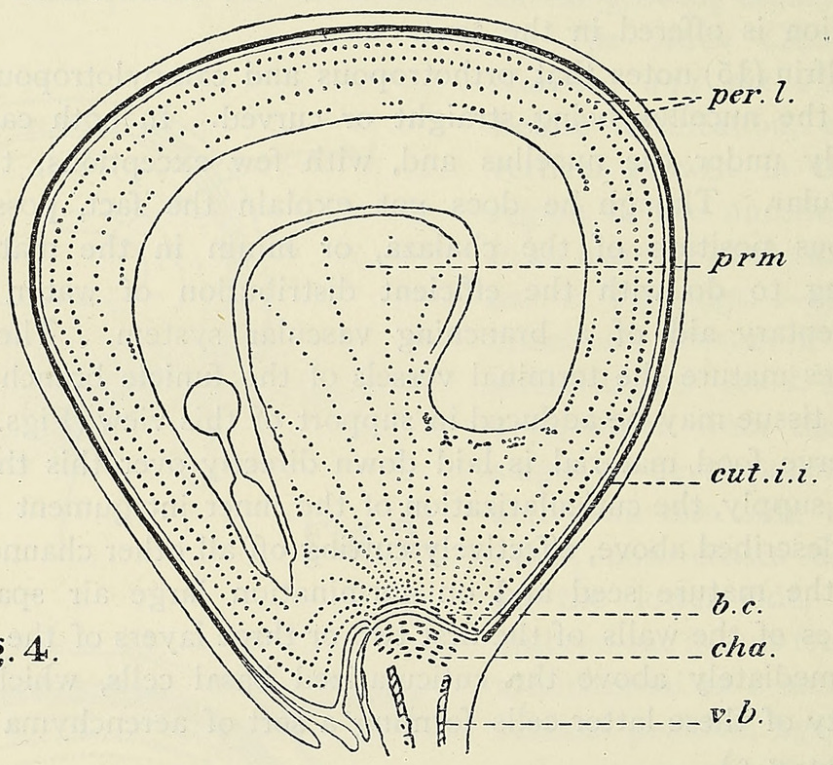


Fig. 4.

prepares itself for solution,' but that is perhaps rather an arbitrary method of description. Before fertilization, the whole nucellar tissue is homogenous. After fertilization, starch localization takes place, which, as we have seen, affects certain cells of definite layers ; but the fact that the layers digested during the growth in length of the embryo-sac are not specialized as storage tissue, hardly justifies one in saying they are 'prepared for solution.' Available for solution would be an expression more consistent with the facts, as the layers in question differ in no sense from the peripheral nucellar layers, which are constantly digested during the growth of the embryo-sac.

Peripheral layers of the nucellus. The peripheral layers of the nucellus are four to five cells thick before (Pl. V, Figs. 6 and 7, *per. l.*) and just after

fertilization (Fig. 16, *per. l.*, Fig. 18, *per. l*). The growth of the embryo-sac takes place at the expense of the inner peripheral layers, which are successively absorbed, and a disorganized row of cells always surrounds the embryo-sac with the exception of a small concave area near the chalaza where the perisperm is developing.

By constant periclinal and anticlinal divisions of the epidermal layer, the peripheral tissue keeps pace with the growth of the ovule, remaining about four or five layers thick. As the growth of the ovule becomes stationary, the meristematic activity of the epidermal layer relaxes (Pl. V, Fig. 19, *per. l.*) until in the mature seed (Pl. VI, Fig. 22, *per. l.*) the external layer alone survives. The cells of this layer increase in size and starch contents, but as their nuclei and cytoplasm remain active, they apparently retain the function of transitory starch storage tissue, which characterizes these peripheral layers from the fertilization stage. This epidermal layer in the seed persists till germination (Figs. 24 and 25, *per. l.*).

Suspensor. The suspensor is filamentous, consisting of one large cell, and succeeded by a varying number of smaller cells in vertical succession, never less than four, generally five or six (Pl. V, Figs. 14–18, *sus.*). The large basal cell (directed towards the micropyle) is formed immediately by the oospore, which elongates considerably after the fusion of the male and female nuclei (Fig. 9, *oosp.*). The resulting nucleus occupies the lower portion of the cell, surrounded by a dense reticulum. The upper portion then elongates, the cytoplasm becomes less dense, until in the extreme apex it completely loses its granular appearance and consists of a densely staining homogeneous substance (Fig. 9, *haus.*). The upper portion of the oospore in this plant elongates so much that it forms a haustorium at the micropylar end, which projects beyond the embryo-sac into the nucellar tissue. A certain compression is traceable in longitudinal section where the embryo-sac terminates (Pl. V, Figs. 9 and 15, *haus.*). In this tip the homogeneity of the contents remains distinct, and the wall is thicker in consistency.

The process is similar in other species of the Alsinoideae, in which the suspensor is not prolonged beyond the embryo-sac. The oospore enlarges and forms the basal suspensor cell, but the apex remains rounder, though it stains darker than the rest of the cytoplasm (Pl. V, Figs, 10, 11, *b. sus. c*). The enlargement of the oospore occurs very quickly. After the first division of the oospore to form the primary suspensor a large vacuole appears, at the end of the basal cell (Figs. 10, 11, 14, 18), and the nucleus stations itself just below it. This position is characteristic and persistent. The contents of the basal cell are extremely dense, the chromatin being lumped in the meshes of the reticulum. The nucleus is very large and active in appearance, and the cell suggests an absorbent organ.

After the first divisions of the primary suspensor the synergidae appear

contracted and empty and are not traceable further, owing to the rapid aggregation of nuclei and dense cytoplasm at this portion of the embryo-sac.

The fan-like arrangement of the cell rows at the apex of the nucellus indicates a convergence towards a given point (Figs. 16–18, *ap. nuc.*), and moreover these cells show disintegration in proximity to the base of the suspensor, and they are arranged with their long axes directed towards it. As the embryo grows the basal suspensor cell elongates (Pl. V, Fig. 19, *b. sus. c.*) and the contents become less dense and more granular, until finally, as the cotyledons develop and the organization of the endosperm cap follows, the suspensor is completely re-absorbed by the latter (Fig. 21, *dis. sus. c.*). The basal cell of the suspensor would thus form the first sucking organ, but, as the wants of the embryo increase, it is replaced by the endosperm cap, with its more complex organization and advantageous position, with regard to actual and potential food supply.

In the Alsinoideae a complete series is obtained in the grades of organization of the basal suspensor cell. In the Alsineae it reaches its greatest development, and in *Stellaria media* the climax may be said to occur. In the Sperguleae it is so reduced as to be hardly differentiated from the rest of the cells of the filament. The importance of this cell is indicated by its complex organization before the first division of the oospore (Fig. 9, *oosp.*).

Most work on the subject seems to point to the fact that the suspensor where it occurs is an absorbent organ. It may produce vermiform haustoria which seek available sources of food supply, as Treub (13) first described for orchids, or it may consist of large swollen cells charged with nutritive material, as in some Leguminosae (Guignard, 17). In the Alsineae the suspensor is very small if we exclude the first cell, consisting of only one row of three or four cells. In the Sperguleae it is more massive, and the cells divide again to form a double row; so that possibly the formation of the large basal cell, where it occurs, is to reinforce the absorbent power of the suspensor as a whole, just as the peculiar development of the micropylar and absorbent portion of this cell in *Stellaria media* suggests an attempt to increase the area of available food supply.

The inner integument. The integuments each consist, as has already been explained, of two layers of cells, forming four layers in all, of which three only persist in the ripe seed. These layers are at first undifferentiated in the case of each integument (Fig. 6, *o. i.*, *i. i.*). In the inner integument which arises first the cells at the apex increase in size as it closes over the nucellus, and these cells project far beyond the outer integument (Fig. 6, *i. i.*). Before fertilization they stain rather darkly (Fig. 7, *i. i.*), and after the passage of the pollen-tube they lose contents and shrink in size often leaving quite a cavity in which the tube persists. They

finally shrivel and close up (Fig. 18, *i. i.*) till in the mature seed the mycropyle is so pressed against the hilum that the outer integument practically closes over it (Pl. VI, Fig. 22). In the young ovule (Pl. V, Fig. 6, *i. i.*) the two layers are still distinct, the individual cells composing them showing centrally placed and active nuclei. As the ovule increases in size they become very much stretched (Fig. 7, *i. i.*), and after fertilization the dividing walls are more or less obliterated, the cells lose protoplasmic contents, and the nuclei disintegrate, the two layers practically fusing into one. ('Nährschicht' of Holfert (20).) The inner wall of layer two abutting on the nucellus assumes a wavy outline of highly refractive appearance. (Pls. V and VI, Figs. 21 and 22, *i. i.*) This inner wall immediately after fertilization becomes cuticularized in conjunction with some of the basal layers of the nucellus as already described on p. 36 (Fig. 18 *b. c.* and *i. i.*).

Outer integument. This consists also at first of two undifferentiated layers (Fig. 6, *o. i.*) but the cells of layer 1 soon increase in size and the nuclei drop to the base of the cell (Fig. 7, *o. i. i.*). After fertilization their walls begin to thicken and grow out but are not cuticularized until maturity (Fig. 19, *o. i.*). They finally form projecting papillae, the surface of the walls showing warty projections (Fig. 22, *o. i.* 1. *sec. pap.*) or small secondary papillae.

The second layer is composed of small cells with active centrally placed nuclei and denser contents (Fig. 7, *o. i.* 2). It suggests a transitory proteid layer and remains distinct till the embryo is well advanced (Fig. 21, *o. i.* 2), after which the contents gradually disintegrate, the cell-walls become crushed against the outer layer and apparently they merely increase the mechanical functions of the latter at maturity (Fig. 22, *o. i.* 2). ('Pigmentschicht' of Holfert (20).) The layers of the outer integument contain starch even in the germinating seed. Tannin is present in the cells of the integuments and the hilum.

This tannin seems characteristic of the moribund cells of the tegumentary layers which at maturation are purely protective.

Balfour (27) puts forward the suggestion that in non-vascular seeds the integuments form the water supply of the ovule. In the case of *Stellaria media* the only possible source of water supply is through the chalaza, the integuments being cut off at a very early stage by the cuticularization of the inside wall of the inner integument abutting on the nucellus. The base of the integuments, however, are in connexion with the chalaza (Pl. V, Fig. 12, *i. i.*), and the possibility of layer 2 of the outer integument forming a sort of water jacket to the growing ovule is suggested by the fact that in *Spergula arvensis* the wing which surrounds the ovule in the plane of the embryo is formed entirely from the proliferation of the cells of this layer (Pl. VI, Fig. 23 *o. i.* 2). These cells contain a little starch, their protoplasmic contents are not marked, and the nuclei are small. They

certainly do not form a proteid reserve, and they are too active in appearance not to suggest some necessary function. The proliferation occurs on the first divisions of the suspensor. On maturity the whole wing dries up forming merely a means for the dispersal of the seed. The differentiation of this wing at such an early stage would support the idea of transitory water storage, the later function to facilitate dispersal being only a secondary result of the transitory nature of the first.

If we take this view in the case of *Spergula arvensis*, there seems no reason not to apply it to the morphological representative of this tissue in *Stellaria media* where it is reduced to one layer which would thus form a specialized water jacket and not a transitory proteid reserve. This would bring the whole question into line with Balfour's apposite suggestion and seems to be the view borne out from the ontogenetic standpoint.

Chalaza. The chalaza is composed of small polygonal cells with large nuclei and dense homogeneous contents. These cells are in direct continuity with the nucellar axile cells and also with the layers of the integuments (Pl. V, Fig. 12, *cha.*). The vessels of the funicle abut on this tissue, branching as the ovule increases in size (Pl. V, Figs. 12, 18, 19, *v. b.*). In early stages it gives a xanthoproteic reaction. Before the funicle breaks off the cells become impregnated with tannin, and after rupture takes place it is bent up against the micropyle forming the hilum (Pl. VI, Figs. 22 and 24, *h.*).

Germination of the Seed.

Cerastium perfoliatum.

Germination begins by the elongation of the cotyledons into the central mass of perisperm, thus forming the first twist of a spiral (Pl. VI, Fig. 24). This elongation was observed in one or two cases in the mature seed, but is exceptional before actual germination, or hydrolysis of the starch reserves takes place.

In this stage the axile cells of the nucellus are elongated, and press laterally on the region between the hypocotyl and the cotyledons (Pl. VI, Fig. 24, *ax. c.*). They show a marked decrease in starch contents in the vicinity of the endosperm cap, the cells of which, on the axile side, practically form part of the nucellar tissue. The chalazal cells are almost obliterated, but the cuticularized layers of the 'aerenchyma' are apparent, large air spaces occurring in the nucellar cells immediately above them (Pl. VI, Fig. 24, *a. s.*). Transverse sections best show the intimate relation of the endosperm cap to the nucellus and root apex of the embryo. The activity of the endosperm is greatest in the first stage of germination, starch appearing in the epidermal cells of the embryo as soon as the growth of the cotyledons begins. A transverse section through the root cap shows the procambial strand (Fig. 25, *pc. s.*), the cortex, the outer layer of which

is densely filled with starch contents (Fig. 25, *c.*), the small cells of the root cap (Fig. 25, *r. c.*), succeeded by the enveloping layer of the endosperm cap cells (Fig. 25, *end. c.*) which present an actively secretory appearance and are connected as one tissue with the root of the embryo on one side, and the nucellus on the other. A section taken above the root cap still shows the endosperm cap cells (Fig. 26, *end. c.*), but in a section through the hypocotyledonary portion they are no longer seen (Pl. VI, Fig. 28).

The second stage in germination which occurs in a day or two according to the temperature and amount of moisture present, is marked by the apical portion of the endosperm being pushed slightly through the micropyle by the root apex on the elongation of the hypocotyl (Pl. VI, Figs. 31 and 32, *end. c.*). It is ruptured immediately (Fig. 33, *end. c.*) as the root grows through it, but the extruded portion which invests the hypocotyl as a collar persists on the seed coat after the cotyledons have been withdrawn (Fig. 35, *end. c.*). The basal portion remaining in the ovule is fused to the nucellus (Fig. 29, *end. c.*).

The cells of the ruptured endosperm cap lose contents and become vacuolated, they also elongate in a lateral direction (Pl. VI, Fig. 30, *end. c.*), but remain in connexion with the few strands of much compressed and empty axile cells which form the remains of the nucellus (Fig. 29, *end. c.* and *ax. c.*). The hypocotyl elongates with extraordinary rapidity and the development of root hairs being more or less simultaneous with the rupture of the endosperm cap (Figs. 33, 34, *r. h.*). In one case they were formed when the root was still in the micropyle. These facts seem to point to the conclusion that once water absorption can take place the embryo is independent of the ovule, though the whole cotyledonary portion may be still enclosed in the seed coat, for it can then utilize the starch which has been transferred to its tissues during germination.

The cotyledons have no connexion with the food reserves of the ovule. The epidermal layer is cuticularized on germination, when it reacts to iodine and sulphuric acid. Stomata appear on the dorsal and ventral surfaces as the hypocotyl emerges (Fig. 41). Air spaces occur in the mesophyll and a thread-like system of vascular strands pervades the lamelli of the cotyledons (Figs. 39 and 39 *a*, *tra.*).

These strands terminate below the apex of the lamelli in two pencil ends of tracheides which lie under a well-marked epithem composed of loose cells, large *water stomata* occurring in the epidermis on the dorsal side (Fig. 42, *w. sto.*).

In some cases where seeds were germinated in the dark with excess of moisture these stomata were more numerous, the epithem better developed, and the tracheids in greater number and more diffused.

The epithem region shows differentiation in the mature embryo, staining lighter than other portions. On germination it remains free of

starch, but the water stomata were not apparent till the formation of the root hairs, therefore not until the emergence of the hypocotyl, when root pressure would first be felt and some organization to regulate osmotic pressure must be called into play. The cotyledons are therefore perfect foliage leaves, the organization of which is complete long before they emerge from the seed coat.

The root apex of the embryo shows well-marked stratification in the mature seed with the root cap differentiated. Starch appears in the columella of the root cap after the differentiation of the root hairs (Fig. 34, *sth.*). This starch is localized to a few central cells of the columella and is evidently statolithic in function, as the grains are relatively few in number and only occur towards the base of the cells in normally growing seedlings.

Specific Differences.

Sperguleae. *Spergularia rubra* and *Spergula arvensis.*

Specific difference is rather marked in the Sperguleae, and runs in one or two lines more suggestive of a primitive form than variation from type.

In both *Spergula arvensis* and *Spergularia rubra* the nucellus is very much curved, bringing the micropyle almost in contact with the funicle, simulating an anatropous ovule. This peculiarity may be due to the greater packing of the ovules in the ovary, in *Spergularia rubra* especially they are extremely numerous.

The layer 2 of the inner integument shows an especial modification in *Spergula arvensis.* In the region of the endosperm cap and continued above it for a certain distance this layer consists of small and active-looking quadrate cells (Fig. 23, *i. i.* 2) which become strongly cuticularized, preserving their well-marked outline both in the mature and germinating seed (Pl. VI, Fig. 30, *i. i.* 2).

Layer 1 of the inner integument is indistinguishable. Over the rest of the ovule the inner integument behaves in the usual manner, viz. both layers fusing owing to the breaking down of the connecting walls through the extreme stretching they undergo to keep pace with the growing ovule (Figs. 23 and 29, *i. i.*). In *Spergula arvensis* layer 2 of the outer integument also undergoes modification, a local hypertrophy of tissue caused by the proliferation of the cells of this layer forming a wing all round the ovule in the vertical plane of the embryo (Fig. 23, *w.*). This wing is composed of small polygonal cells elongated in longitudinal section with very small nuclei and thin contents. As the ovule matures this tissue dries up, the empty cells remaining as an investing wing; it therefore serves a secondary function as a mechanism for wind dispersal. A water jacket may possibly be the first function of this proliferation of tissue. The appearance of the cells suggests water storage in the absence of dense staining contents which would characterize proteid preserves. They also

contain isolated starch grains which would not be traceable in cells containing proteid material. This wing is differentiated while the embryo is still in the suspensor stage.

The primary suspensor cell in both *Spergula arvensis* and *Spergularia rubra* shows a well-marked reduction in size. This is so apparent that Hegelmaier (10) speaks of the small celled suspensor of *Spergula arvensis*. Schleiden and Vogel (2) figure a row of undifferentiated cells of *Spergula pentandra*. This species was not available for examination in the present case, but in both the species investigated the primary cell is still considerably differentiated in size and contents from the succeeding ones of the row. The suspensor in the Sperguleae has a tendency to become more massive, as the cells composing it divide again vertically, thus forming a double row of cells. The primary cell does not divide again. The air spaces in the angles of the walls of the cells occurring just above the 'aerenchyma' layers are very pronounced in the mature and germinating seed of *Spergula arvensis*.

The synergidae are small and short in *Spergula arvensis* and long and narrow in *Spergularia rubra*.

Antipodals are well marked in both.

Alsineae. The synergidae fall into two types—

1. Long, with large nuclei, which attain their greatest development in *Arenaria trinervia* (Fig. 8, *syn.*) and *Stellaria media*.

2. And a shorter type with inconspicuous nuclei, which occurs in *Sagina procumbens*, *S. apetala*, and *Stellaria uliginosa*.

Antipodals are not always present or at least are not sufficiently obvious to be observed. They are well seen in *Sagina procumbens* and *S. apetala*, and in *Stellaria media*. In other species though clearly shown in the progressive free nuclear divisions of the embryo-sac nucleus, they were not so apparent at a later stage.

Definitive Nucleus. In all species, with the exception of *Stellaria media*, there is no actual contact between the nuclear membranes of the oosphere and definitive nucleus, some cytoplasm always separates them (Fig. 8, *oos.* and *d.n.*).

Basal suspensor cell. In *Cerastium* and *Stellaria* species this cell is very large. In *Sagina apetala* and *S. procumbens* it is smaller. It can be recognized in all the species before the division of the oospore, and the apex, though only prolonged as an haustorium in *Stellaria media*, shows the same marked differentiation in contents which stain darker and more homogeneously in that region.

Persistent pollen-tube. Is characteristic for all species except *Stellaria media*, and is especially well seen in *Sagina apetala* and *S. procumbens*, *Cerastium* species, and *Stellaria aquatica* (Fig. 13). The pollen-tube twists on itself before it enters the synergidae, forming a plug on the apex

of the embryo-sac, with which it is so intimately fused that it can always be dissected out still attached to the apex of the latter. The wall of the pollen-tube is very thick and the contents become granular. The tubes are well differentiated in Heidenhain's Iron Alum Haematoxylin.

Seed Coat. Shows distinct specific variation. The degree of cuticularization, the form and number of cells which enlarge and the degree of tannin formation seem to be constant characters. Yet even in this case variation is more apparent than real, depending chiefly on the papillose manner in which the cell-wall grows out, and the nature of the secondary projections which occur in it. In *Stellaria aquatica* the cells of the outer integument grow out broadly, using up their whole diameter. The ovule in consequence appears covered with papillae as the projecting cell-walls are almost in contact. In *Cerastium perfoliatum* only the immediate portion of the wall in the centre of a cell is raised, and radiating projections round the surface of the ovule result (Fig. 24, *o. i.* 1). The same remarks hold for *Spergularia salina* (Fig. 32, *pap.*). *Stellaria media* approximates more to *Stellaria aquatica* in the form of outgrowth of the individual cell-walls, but secondary warty projections occur in the wall of each cell (Fig. 22 *a*, *sec. pap.*). These projections in *Spergula arvensis* form large secondary papillae, one of which may grow out from each epidermal cell or in some cases only from a limited number of epidermal cells (Fig. 23, *sec. pap.*). In the so-called *id.* var. *sativa*, these projections are altogether absent, which, as the numbers are inconstant in the type, seems to hardly justify sub-specific distinction.

In both *Spergula arvensis* and its so-called var. *sativa* the wall has a wavy cuticle which in *Spergula arvensis* proper is continued in the same form on the secondary papillae (Pl. VI, Fig. 23, *pap.*).

Abnormalities.

In *Stellaria Holostea* a case of two megaspores in one ovule was seen (Pl. VI, Fig. 36, *m.*).

In *Cerastium glomeratum* two nucelli were observed in one ovule, each nucellus with a perfectly developed embryo-sac in the definite nucleus stage. As this abnormality has been fully described and figured in a previous paper (29) it is only referred to here.

In *Sagina procumbens* a very interesting case of vegetative outgrowth of the nucellus is figured on Pl. VI, Fig. 37. It occurs in a microtome series of the ovary and can be traced through four sections. No embryo-sac formation is apparent, the nucellus consisting of very actively dividing small cells, quite different in shape and contents from the tegumentary tissue of the ovule. It projects well beyond the integuments, which are not normally developed. In Fig. 37 the section is oblique, for while showing

the outgrowth of the nucellus best, it cuts into the cells of the outer integument on the posterior side of the ovule.

Conclusions.

The general result of work done on the Caryophyllaceae, section Alsinoideae, emphasizes the view that it is a very well-defined group of plants, the members being characterized by great uniformity both in the morphological development of the sporophyte and in histological structure. This uniformity extends to the reproductive organs, and investigation on the Alsinoideae in this direction tends to show that apart from more specific differentiation there is a certain developmental trend in the direction of greater specialization from the Sperguleae to the Alsineae.

If we pass in review the results obtained, the three most important points seem to be—1, the organization of the ovule in relation to the passage and storage of food supplies for the embryo; 2, the manner in which such food supplies are rendered available; and 3, the indication of certain lines along which development has proceeded within the group.

1. *The organization of the ovule.* The ovule in its complete form consists of the chalaza, a large nucellus with embryo-sac, invested by two integuments, and each of these component parts stands in important relation to the development of the whole.

The chalaza is the seat of elaboration of proteid material, and the whole of the organized food supplies required for the growth of the ovule and embryo, together with water and air, must pass through this tissue. It is situated in a very advantageous position, abutting on the vascular system of the funicle which branches freely into it during the later stages of development, whilst on the distal side its cells are in serial connexion with the axile rows of the nucellus. The perisperm is laid down in the upper region of these axile rows. Laterally, the chalaza is in communication with the integuments. The medium of diffusion between the chalaza and the nucellus would appear to be a few of the basal layers of the nucellus, the walls of which become cuticularized after fertilization and show shallow pits. In the mature seed and on germination large air spaces occur in the angles formed by the walls of several layers of the unmodified nucellar cells immediately above the cuticularized layers. This fact is suggestive of a possible function for these basal layers as a species of aerenchyma. That all gaseous interchange must necessarily be limited to this ' aerenchyma ' is shown by the early cuticularization of the inner wall of layer 2 of the inner integument which effectually cuts off every other source of supply.

The immediate elongation of the embryo-sac which follows closely on fertilization, and its subsequent enlargement in the vicinity of the chalaza, is

also possibly correlated with the differentiation of the 'aerenchyma' in relation to the supply of water and oxygen.

We have seen that the integuments consist each of two layers which, in the case of the inner integument are undifferentiated, the cells of the apical portion merely increasing in size where they project beyond the outer integument. This part is subsequently used up during the passage of the pollen-tube. Over the periphery of the ovule they lose their cell contents, and become so stretched as growth goes on that the dividing walls disappear, leaving apparently one layer only.

The outer integument is composed of two differentiated layers, layer 1 being purely protective, increasing its area by the papillar outgrowth of the cells forming it and its mechanical function by the cuticularization of the cell-walls. It is possible that layer 2, the cells of which remain active and functional till maturity by dividing to keep pace with its growth, may act as a water jacket, forming a sort of transitory water storage tissue for the growing ovule. This hypothesis is strengthened by a comparison with the mode of its development in some of the Sperguleae. In these plants a proliferation of the cells of the layer under discussion results in a local hypertrophy, ultimately forming a wing which extends round the ovule in the vertical plane of the embryo, but in its earlier stages is very suggestive of a transitory water storage function.

The nucellus is differentiated into two regions, viz.: i. The peripheral layers, which are available for solution by the cytoplasm of the embryo-sac to provide for increase in size, and which when the latter obtains its maximum growth are gradually reduced to one layer, which persists till the germination of the seed and even in the discarded seed coat. ii. The axile rows which receive and distribute the supplies of food material from the chalaza in their basal portion and elaborate the starch reserves or perisperm in the upper cells of these rows, the cells increasing greatly in size as the starch is laid down.

2. *The manner in which the food supplies are made available.* That this occurs in the first place through the agency of the suspensor is suggested by the remarkable form assumed by the latter owing to the great differentiation of the basal cell of the filament. The early differentiation of that cell points to the same conclusion since it assumes its final shape even before the first division of the oospore (Pl. V, Fig. 9, *oosp.*).

The persistence of the pollen-tube, and the characteristic plug formed by it on the apex of the embryo-sac, may also be interpreted as corroborative evidence for the activity of the suspensor, as the channel formed by the pollen-tube in its passage through the nucellus is kept open, thus increasing the area available for solution. In *Stellaria media*, where the tube is not persistent and does not form a plug, the plant has overcome the difficulty in reaching the apical nucellar tissue by sending an haustorium

from the primary cell itself into this tissue. The function of the suspensor as a sucking organ would seem to be limited to the period preceding free-cell formation to the endosperm. As the basal cell elongates during the growth of the embryo the contents become less dense and more granular, and it finally remains as a mere empty sac.

The endosperm replaces the suspensor as the cotyledons are differentiated in the growing embryo, and we have seen that it is the apical portion alone which functions as a secretory organ, in the form of a cap composed of a single layer of cells, which invests the radicle. The endosperm is thus differentiated into two portions, structurally as well as functionally diverse—an active apical region, composed of small cells with dense contents, and an inactive peripheral portion with large and vacuolated cells which are stretched over the remaining surface of the embryo-sac. This differentiation is no doubt correlated with the favourable situation of the apical portion of the embryo-sac, being in immediate vicinity to the perisperm reserves of the nucellus and, through the lower axile cells of the latter, to the water supply through the chalaza.

As the seed matures, we see a further approach to these chief sources of food supply by the gradual pressing of the micropyle against the chalaza, which is characteristic of the maturation stage. In considering the autonomous organization for nutrition in these ovules, it is interesting to refer to the complex outside mechanisms in the case of *Phlox Drummondi* (Billings, 24), where in early stages the ovary wall is described as forming the starch reserve. A channel for the passage of food supply to the embryo is provided in the form of a papillose outgrowth of the ovary wall, in the vicinity of the micropyle ; this papilla presses against the latter, which becomes closed and serves as conducting tissue.

The organization of the secretory cells of the endosperm in the Alsinoideae is very complete. They form an investing cap surrounding the radicle, which grows down into them, and completely fuse with the nucellus, forming an intimate connexion between it and the embryo which is only ruptured by the elongation of the hypocotyl on the germination of the seed. Even then their connexion with the nucellus is not affected, and they remain attached to the few strands of tissue which have not been absorbed by their agency for the benefit of the embryo sporophyte. In some chance sections through a seed, where a fungus mycelium had consumed the endosperm, evidently not being able to attack the perisperm, the embryo was malformed and undeveloped, with two straggling cotyledons composed of a few strands of tissue, the whole limited to the apical portion of the sac, thus pointing to the endosperm as the one agent for the supply of proper food and directive energy. Seeds from which the endosperm was artificially removed did not germinate. The results of the present investigation, as far as the Alsinoideae group of the Caryophyllaceae are

concerned, thus bear out Johnson's suggestion that the restriction in the formation of the endosperm to that of a purely digestive tissue which he observed in certain Piperaceae, obtains in all seeds with abundant perisperm, such as Chenopodiaceae, Polygonaceae, and Caryophyllaceae, but he goes on to say—

'Observations thus far lead me to believe that in the perisperm-containing seeds mentioned the embryo-sporophyte of the second generation is never nourished by the parent sporophyte directly, but always through the intermediate gametophyte.'

This view, as far as the Alsinoideae are concerned at least, only holds with regard to the ultimate organization of the embryo and the germination of the seed.

Before the cotyledons are differentiated everything points to the food material being digested and passed through the suspensor, and the embryo is accordingly nourished by the parent sporophyte up to that stage. It is only after the organization of the endosperm cap nuclei into a definite layer of cells that a portion of the endosperm takes on the function of secretory agent, which it retains till germination, when it is ruptured by the radicle on the elongation of the hypocotyl. A more limited function than is described by Johnson for the endosperm in some of the Piperaceae thus results from increased complexity and economy in organization; and the jacketing by the endosperm of the undifferentiated embryo at germination, which is such a striking feature in the Piperaceae, is reduced in the Caryophyllaceae to the short period necessary for the transference of the starch reserves in the perisperm to the tissues of the embryo on germination. The endosperm cells in this order fuse up more or less completely with the nucellus, and remain attached to the tissue of the latter when they lose connexion with the embryo, which is completely organized in the mature seed. There is certainly an elongation of the cotyledons in the seed prior to that of the hypocotyl, but the cotyledons in this stage are complete leaves, with a vascular system, cuticularized epidermis, stomata, and air spaces, and they are also provided with an epithem tissue and water stomata at their apices.

3. *Trend of Development.* In the Alsineae there seems to be a slight tendency towards greater specialization and development on the Spergulean type. The more massive and shorter suspensor occurring in the latter, with its small basal cell, is replaced by what may be a more labile filamentous one, in which the basal cell is greatly developed for absorption purposes, even to the producing of an haustorium as in *Stellaria media.*

If in the development of the integuments we look upon the wing which characterizes some of the Sperguleae and results from the local hypertrophy of cells of layer 2 of the outer integument, as primarily functioning as a water jacket, but subsequently becoming modified for wind dispersal on

the drying up of the cells composing it, we get a mechanism in which the latter function is often at a discount. In *Spergularia salina* winged and unwinged seeds occur in the same ovary (Pl. VI, Figs. 31, 34). In other species the same condition obtains, but more exceptionally. This fact suggests that water storage is the determining factor in the proliferation of this tissue, and where the supply of H_2O is deficient, or unequally distributed, the process of formation is interrupted. Therefore we might consider the local hypertrophy which is the origin of this band of tissue to be entirely suppressed in the Alsineae, and look upon the specialized layer of very active cells, capable by division of keeping pace with the growing ovule, and possibly of regulating water storage, as an advance in organization.

Finally, then, everything seems to point to the conclusion that the Alsinoideae are members of a very old and stable family. On one side they suggest an intermediate stage in the development of the ex-albuminous seed by a progressive reduction in the functions of the nucellus. The correlative increase in the activity of the endosperm results in the reserve food material being stored in the embryo itself through the medium of the latter tissue. M. Péchoutre's researches on the Rosaceae seem to point to that family as providing further illustration of the same tendency.

Among the points of comparison afforded by the Rosaceae may be mentioned the functional rôle played by the endosperm where some portion persists in the ripe seed.

The endosperm in this family is characterized by a limiting peripheral proteid layer (assize protéique), distinguished by abundant proteid reserves, but not otherwise differentiated from the other layers. In all cases some of this tissue persists in an active condition in the ripe seed. The conclusion drawn by Péchoutre that the function of this tissue is not mechanical, as in the case of the seed coat to which it is fused, but rather physiological in character, seems to be justified.

In the Alsinoidean stage of development the endosperm is limited to one layer only, and its function is entirely secretory and digestive. When this tissue increases in volume, the outer layer is specialized as a ferment layer, the increase in volume being associated with the increase in activity necessary to the transference of all the reserve food material through the endosperm to the embryo before germination, instead of its being stored in the nucellus to be drawn upon as required. If we consider the other end of the scale and take certain Piperaceae as a starting-point, a great restriction in the function of the endosperm is apparent in the Alsinoideae. In the Piperaceae the embryo at maturity is an undifferentiated mass of cells, and on germination the endosperm extrudes from the seed coat and jackets the embryo till cotyledons, hypocotyl, and root are organized.

In the Alsinoideae the endosperm has no other function beside that of secretion and digestion, and it does not bring these powers into play until

free cell division takes place. The complete organization of the embryo at maturity restricts the necessity for jacketing. It might perhaps be suggested that this restriction which obtains in the Alsinoideae is correlated with the more complete development of the suspensor as a primary digestive agent, and that this, by enabling the embryo to immediately draw on the organized food supplies of the parent, ensures the organization of the embryo being completed within the seed. The endosperm comes into play to supply what is beyond the capabilities of the suspensor as the embryo increases in size, and its function thus both begins before germination and continues afterwards on the same lines. As we get higher in the scale its activity after germination is more and more reduced until finally it is no longer present on maturity. The storage of the starch reserves in the embryo itself is another advance in specialization and economy. A small beginning is indicated by the starch which appears in the epidermal layer of the embryo in the Alsinoideae after germination, when the rupture of the endosperm necessitates the presence of some reserve to ensure continuance of growth till the cotyledons are drawn from the seed-coat and can assimilate on their own account.

In conclusion, I must thank the Curator of the Chelsea Physic Garden for supplying and growing material required for the purposes of this investigation; Mr. Malcolm Wilson, B.Sc., for very kindly collecting various species; and especially Professor Farmer for his unfailing kindness, help, and advice in the course of this work.

Bibliography.

1. Grew: Anatomy of Plants. 1682, Book IV, p. 196, Pl. 63.
2. Schleiden and Vogel: Ueber das Albumen. Act. Acad. Nat. Cur., xix, 1839, pp. 5–96, Pl. 41, Figs. 31–33.
3. Meyen, F. G. F.: Neues System der Pflanzenbiologie. Trans. Ann. Sci. Nat. Bot., xv, 2, 1841, p. 220, Pl. 16, Figs. 14–20.
4. Unger, F.: Anatomie und Physiologie der Pflanzen. 1855, p. 389.
5. Tulasne, L. R.: Nouvelles études d'embryogénie végétale. Ann. Sci. Nat., iv, 4, 1855, pp. 84–9, Pl. 14, Figs. 1–8.
6. Hofmeister, W.: Neuere Beobachtungen über Embryobildung der Phanerogamen. Pringsheim, Jahrb. wiss. Bot., i, 1858, pp. 82–188, Pls. 7–10.
7. Le Monnier, M. G.: Recherches sur la nervation de la graine. Ann. Sci. Nat. Bot., xvi, 5, 1872, pp. 233–304, Pls. 10–12.
8. Warming, E.: De l'ovule. Ann. Sci. Nat. Bot., vi, 5, 1877, pp. 177–266, Pls. 7–13.
9. Vesque, J.: Développement du sac embryonnaire des Phanérogames Angiospermes. Ann. Sci. Nat. Bot., vi, 6, 1878, p. 263, Pl. 11, Figs. 1–7.
10. Hegelmaier, E.: Vergleichende Untersuchungen über Embryoentwickelung dicotyledoner Keime. Stuttgart, 1878.
11. ———————: Untersuchungen über die Morphologie des Dicotyledonen-Endosperms. Nova Acta Leopoldina, 49, 1885, pp. 1–104, Pls. 5.

12. HEGELMAIER, E.: Ueber Orientirung des Keimes in Angiospermen. Bot. Zeit., 53, 1895, p. 163.
13. TREUB, M.: Notes sur l'embryogénie de quelques Orchidées. Naturk. Verh. Koninkl. Akad. Deel, xix, 1879, pp. 1–50, Pls. 1–8.
14. FISCHER, A.: Zur Kenntniss der Embryosackentwickelung einiger Angiospermen. Jenaische Zeitschrift Naturwiss., 14, 1880, pp. 90–132, Pls. 2–5.
15. GODFRIN, M. J.: Étude histologique sur les téguments séminaux des Angiospermes. Bull. Soc. Sci. Nancy, v, 2, 1880, pp. 109–219, Pls. 1–5.
16. WARD, H. MARSHALL: A contribution to our knowledge of the Embryo-sac in Angiosperms. Journ. Linn. Soc. Bot., xvii, 1880, pp. 519–46, Pls. 17–25.
17. GUIGNARD, L.: Note sur la structure et les fonctions du suspenseur embryonnaire chez quelques Légumineuses. Paris, Acad. Sci., Compt. Rend., 91, 1880, pp. 346–9.
18. ———: Recherches sur le sac embryonnaire des Phanérogames Angiospermes. Ann. Sci. Nat. Bot., xiii, 6, 1882, pp. 137–92, Pls. 3, 4.
19. LISTER, MISS G.: On the origin of the placentas in the Tribe Alsineae of the Order Caryophyllaceae. Linn. Soc. Journ. Bot., xx, 1883, pp. 423–9, Pls. 32–5.
20. HOLFERT, J.: Die Nährschicht der Samenschalen. Flora, lxxiii, 1890, pp. 279–313, Pls. 11–12.
21. SARGANT, E.: The formation of the sexual nuclei in *Lilium Martagon*. I. Oogenesis. Ann. Bot., x, 1896, pp. 445–7, Pl. 22–3.
22. NAWASCHIN, S.: Ueber das Verhalten des Pollenschlauches bei der Ulme. Bull. Acad. Imp. Sci. St. Pétersbourg, viii, 5, 1898, pp. 345–57, Pl. 1.
23. JOHNSON, D. S.: On the development of *Saururus cernuus*, L. Bot. Gaz., Sept., 1900, p. 208; Bull. Torr. Club, 27, 1900, pp. 365–72, Pl. 22.
24. BILLINGS, F. H.: Beiträge zur Kenntniss der Samenentwickelung. Flora, 88, 1901, pp. 253–318.
25. JOHNSON, D. S.: The Embryology and Germination of the Genus Peperomia. Abstract Science, 15, 1902, pp. 408–9.
26. ———: On the Development of certain Piperaceae. Bot. Gaz., xxxiv, 1902, pp. 321–40, Pls. 9–10.
27. BALFOUR, I. B.: The Angiosperms. Address to the Bot. Sect. Brit. Assoc. Adv. Sci., Glasgow, 1901.
28. PÉCHOUTRE, F.: Contribution à l'étude du développement des Rosacées. Ann. Sci. Bot., xvi, 8, 1902, pp. 1–158.
29. GIBBS, L. S.: Note on Floral Anomalies in species of Cerastium. New Phytologist, iii, 9 and 10, 1904, pp. 243–7, Figs. 81–4.
30. STRASBURGER, E.: Die Apogamie der Eualchemillen. Jahrb. wiss. Bot., xli, 1905, pp. 88–164, Pls. 1–4.

EXPLANATION OF PLATES V AND VI.

Illustrating Miss Gibbs's paper on the Seed of the Alsinoideae.

PLATE V.

Fig. 1. *Stellaria uliginosa.* Exceptional case of megaspore cutting off tapetal cell: *m.* megaspore; *t.* tapetal cell. × 1100.

Fig. 2. *Stellaria uliginosa.* Tapetal cell showing further anticlinal division: *m.* megaspore; *t.* tapetal cell; *ax. c.* axile cell-layer of nucellus. × 1100.

Fig. 3. *Arenaria trinervia.* Enlarging megaspore: *m.* megaspore; *ax. c.* axile cell-layer of nucellus; *ep.* epidermis. × 450.

Fig. 4. *Stellaria Holostea.* Epidermis showing increased anticlinal divisions at apex of nucellus: *m.* megaspore; *ax. c.* axile cell-layer of nucellus; *ap. nuc.* apex of nucellus. × 1100.

Fig. 5. *Cerastium glomeratum.* First division of embryo-sac nucleus: *e. s.* embryo-sac; *ax. c.* axile cells. × 1100.

Fig. 6. *Stellaria media.* Ovule with embryo-sac showing two polar nuclei: *e. s.* embryo-sac; *oos.* oosphere; *syn.* synergidae; *p. n.* polar nuclei; *ant.* antipodals; *ax. c.* axile cell-layers of

nucellus; *per. l.* peripheral layers of nucellus; *ap. nuc.* apex of nucellus; *st.* starch; *dis. c.* disorganized cells; *i. i.* inner integument; *o. i.* outer integument; *mic.* micropyle; *cha.* chalaza. × 400.

Fig. 7. *Stellaria media.* Ovule with embryo-sac showing polar nuclei fused into one definitive nucleus: *d. n.* definitive nucleus; *o. i.*^{1}, *o. i.*^{2} layer 1 and layer 2 of outer integument; *i. i.*$_1$, *i. i.*$_2$ layer 1 and layer 2 of inner integument; other lettering as before. × 400.

Fig. 8. *Arenaria trinervia.* Embryo-sac showing oosphere, definitive nucleus and synergidae: *oos.* oosphere; *d. n.* definitive nucleus; *syn.* synergidae. × 450.

Fig. 9. *Stellaria media.* Oospore forming basal suspensor cell with haustorium. Definitive nucleus preparing to divide: *oosp.* oospore; *haus.* haustorium; *d. n.* definitive nucleus; *syn.* synergid; *p. t.* pollen-tube. × 1100.

Fig. 10. *Arenaria trinervia.* First segmentation of oospore into two suspensor cells: *b. sus. c.* basal suspensor cell; *end. n.* endosperm-nuclei. × 450.

Fig. 11. *Arenaria trinervia.* Suspensor with three cells: *b. sus. c.* basal suspensor cell; *end. n.* endosperm-nuclei; *syn.* synergidae. × 450.

Fig. 12. *Arenaria trinervia.* Longitudinal section through chalaza and nucellus, showing cuticularized basal cells connecting up with the chalaza and integuments: *nuc.* nucellus; *b. c.* basal cells of nucellus; *cha.* chalaza; *v. b.* vascular bundle of funicle. × 400.

Fig. 13. *Stellaria aquatica.* Apical cells of nucellus prolonged as papillae: *ap. nuc.* apical cells of nucellus; *nuc.* nucellus; *p. t.* pollen-tube; *b. sus. c.* basal suspensor cell. × 400.

Fig. 14. *Stellaria media.* Embryo-sac with embryo, showing aggregation of endosperm-nuclei at apical end (endosperm-nuclei on further side of embryo-sac shaded): *end. c.* endosperm cap; *b. sus. c.* basal suspensor cell; *emb.* embryo. × 450.

Fig. 15. *Stellaria media.* Next section in same series showing haustorium of basal suspensor cell protruding beyond embryo-sac: *haus.* haustorium; *b. sus. c.* basal suspensor cell; *e. s.* embryo-sac. × 450.

Fig. 16. *Cerastium glomeratum.* Longitudinal section through ovule: Embryo-sac elongating after fertilization: *per. l.* peripheral layers of nucellus; *b. c.* basal cells of nucellus; *e. s.* embryo-sac; *prm.* perisperm; *ax. c.* axile-cells. × 110.

Fig. 17. *Sagina apetala.* Longitudinal section of apex of ovule, showing persistent pollen-tube: *p. t.* pollen-tube; *p. t. t.* pollen-tube twist; other lettering as before. × 400.

Fig. 18. *Stellaria media.* Longitudinal section of ovule with embryo, showing perisperm formation in nucellus: *prm.* perisperm; *emb.* embryo; *sus.* suspensor; *fun.* funicle; *sus.* suspensor; other lettering as before. × 400.

Fig. 19. *Stellaria media.* Longitudinal section of ovule with heart-shaped embryo showing progressive perisperm formation in nucellus and reduction of peripheral layers: *cots.* cotyledons; other lettering as before. × 110.

Fig. 20. *Stellaria media.* Longitudinal section of ovule, showing embryo with cotyledons and endosperm cap; lettering as before. × 75.

Fig. 21. *Stellaria media.* Apical portion of same section under higher magnification, showing root apex of embryo, free-cell formation in endosperm cap and disorganizing suspensor; also cells of layer 1, outer integument, enlarging as papillae: *dis. sus.* disorganized suspensor; other lettering as before. × 110.

PLATE VI.

Fig. 22. *Stellaria media.* Longitudinal section of mature seed showing embryo, endosperm cap, one persistent peripheral layer and central perisperm mass of nucellus with cuticularized basal cells and air spaces above them: *pl.* plumule; *pc. s.* procambial strand; *sec. pap.* secondary papillae; *a. s.* air spaces; other lettering as before. × 110.

Fig. 22 *a.* Longitudinal section of the wall of a cell of layer 1 of the outer integument showing secondary papillae and wavy cuticle, after treatment with Eau de Javelle and I & H_2SO_4: *sec. pap.* secondary papillae; *cut.* cuticle. × 450.

Fig. 22 *b.* Surface view of wall treated in the same way: *sec. pap.* secondary papillae; *cut.* cuticle. × 450.

Fig. 22 *c.* Longitudinal section of basal cells of nucellus, showing shallow pits in the cuticularized walls: after treatment with Eau de Javelle and I & H_2SO_4. × 450.

Fig. 23. *Spergula arvensis.* Transverse section of ovule showing embryo-sac cut through the cotyledonary and hypo-cotyledonary portion, the proliferation of layer 2 of the outer integument, forming a wing of tissue round the ovule in the plane of the embryo, and secondary club-shaped papillae on the cell-walls of layer 1 of the outer integument: *w.* wing; other lettering as before. × 110.

Fig. 24. *Cerastium perfoliatum.* Longitudinal section of germinating seed, showing elongation of cotyledons; *h.* hilum; other lettering as before. × 110.

Fig. 25. *Cerastium perfoliatum.* Transverse section of germinating seed, same stage as Fig. 24, cut through the apex of embryo and nucellus in the root-cap region of the former, showing the sequence of tissues and their relation to the endosperm cap: Testa: *o. i.* outer integument; *i. i.* inner integument (two layers fused); *per. l.* peripheral layer of nucellus; *end. c.* endosperm cap; Embryo: *r. c.* root cap; *c.* cortical layers, outer densely packed with starch contents; *pc. s.* procambial stand. × 450.

Fig. 26. *Cerastium perfoliatum.* Transverse section of germinating seed; same series as Fig. 25, just above root cap; lettering as before. × 450.

Fig. 27. *Cerastium perfoliatum.* Diagram of transverse section of germinating seed showing the radicle of the embryo surrounded by the endosperm cap. × 110.

Fig. 28. *Cerastium perfoliatum.* Transverse section of germinating seed, same series, through hypocotyl of embryo, above the endosperm cap; lettering as before. × 450.

Fig. 29. *Spergula arvensis.* Longitudinal section through germinating seed, showing spiral elongation of cotyledons, extrusion of hypocotyl and subsequent rupture of endosperm cap, with axile cells of nucellus reduced to a few strands: *hyp.* hypocotyl; other lettering as before. × 75.

Fig. 30. *Spergula arvensis.* Same section, cells of endosperm cap under higher magnification, showing small quadrate cuticularized cells of layer 2 of the inner integument, which are only so modified in the apical region of the ovule in this species; lettering as before. × 450.

Figs. 31–34 are from seed which was three years old. Where the endosperm cap extruded slightly through the micropyle (Fig. 31, *end. c.*) the exposed cells had dried up and this portion formed a dark mark on the endosperm cap (Fig. 33, *end. c.*) which was observed on all these seeds.

Figs. 31–34. *Spergularia salina.* Germinating seed from the first extrusion to the rupture of the endosperm cap by the elongating radicle of the embryo to the formation of root hairs on the latter: *pap.* papillae (*formed by outgrowth of some cells of layer* 1, *outer integument*); *w.* wing; *end. c.* endosperm cap; *r. h.* root hairs; *r. c.* root cap; *sth.* statolithic starch; *hyp.* hypocotyl. × 75.

Fig. 35. *Spergula arvensis.* Seedling, cotyledons extruded, the endosperm cap remaining on the seed-coat: *cots.* cotyledons; *r. h.* root hairs; *end. c.* endosperm cap, mag.

Fig. 36. *Stellaria Holostea.* Longitudinal section showing two megaspores in one nucellus: *m.* megaspore; *ax. c.* axile cells; *epi.* epidermis; *ap. nuc.* apex of nucellus. × 1100.

Fig. 37. *Sagina procumbens.* Longitudinal section of ovule showing vegetative outgrowth of nucellus: *nuc.* nucellus.

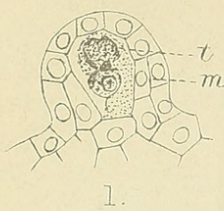
Fig. 38. *Sagina procumbens.* Apex of cotyledon just emerging from seed coat: *epi.* epidermis; *tra.* tracheids.

Fig. 38 *a.* Tracheids. × 450.

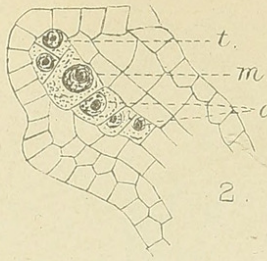
Fig. 38 *b.* Longitudinal section of cells of the epidermis with starch contents: *st.* starch; *cut.* cuticle. × 450.

Fig. 39. Surface view of stoma on ventral surface of cotyledons just emerged from seed coat: *sto.* stoma. × 450.

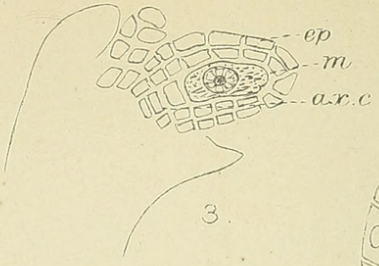
Fig. 40. Surface view of a water stoma surrounded by loose epithem cells, on dorsal surface of cotyledon, just emerged from seed coat. × 450.



1.

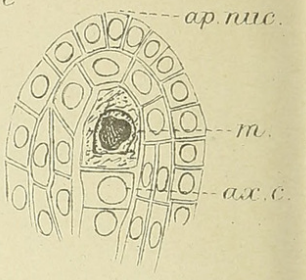


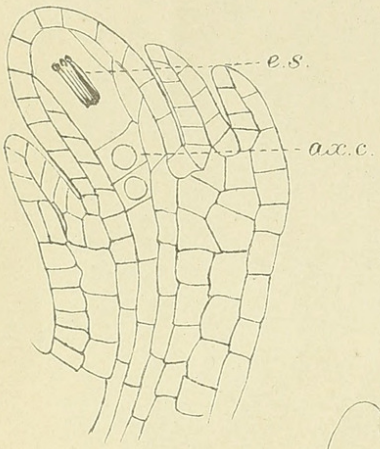
2.



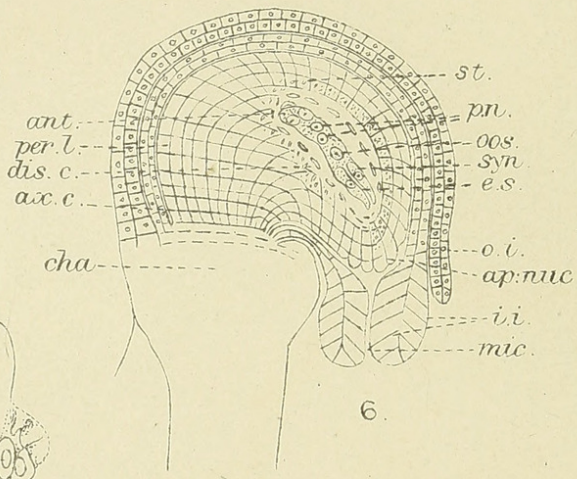
3.

4.



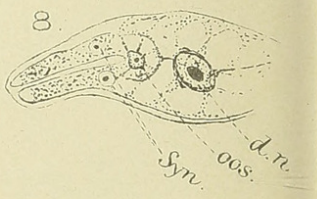


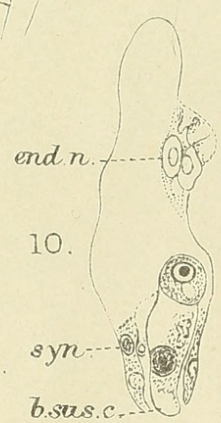
5.



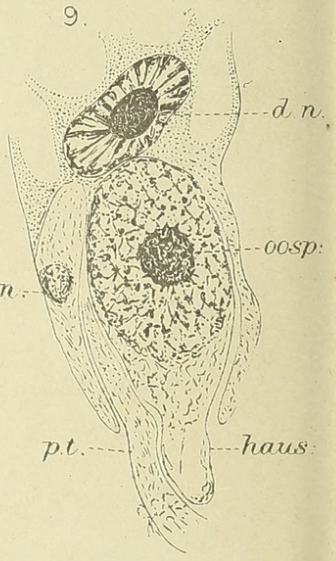
6.

8.

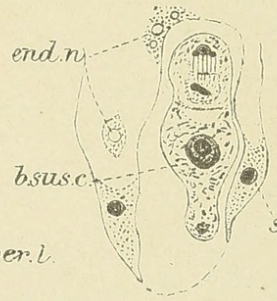


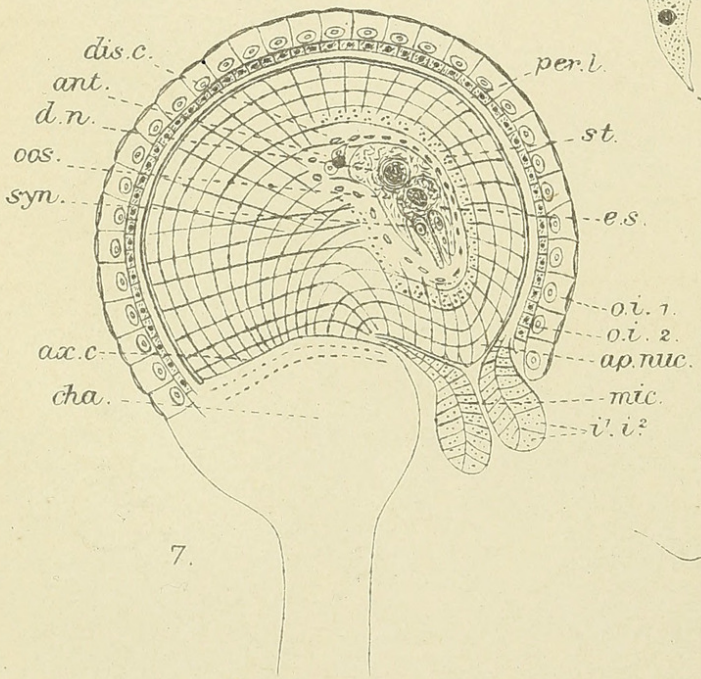


9.

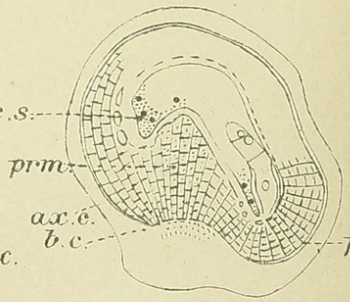


11.

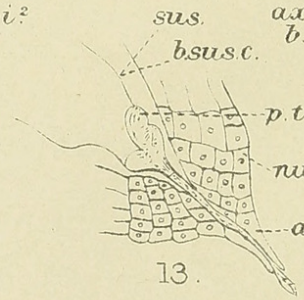




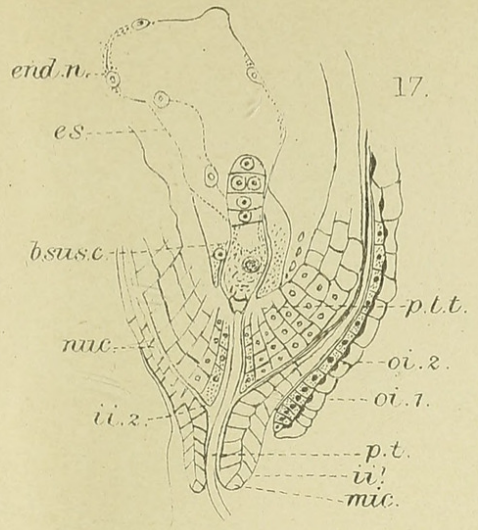
7.

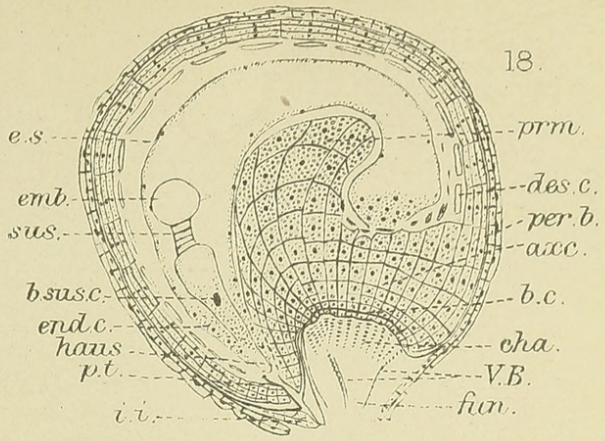


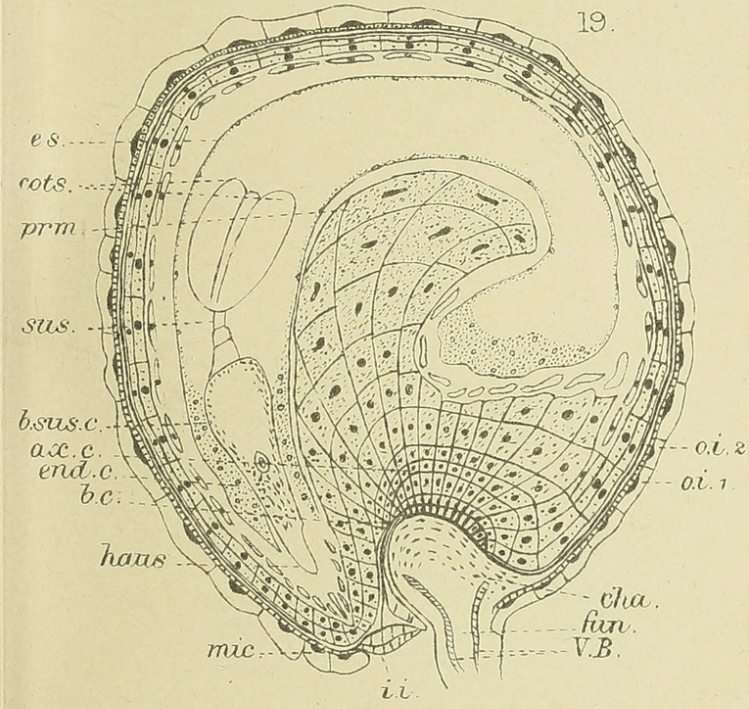
16.

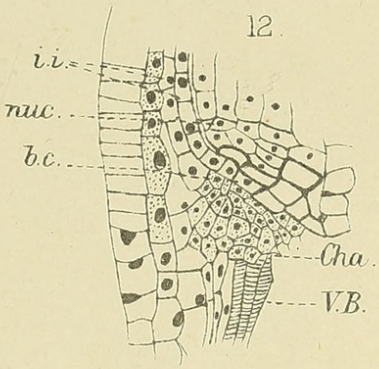


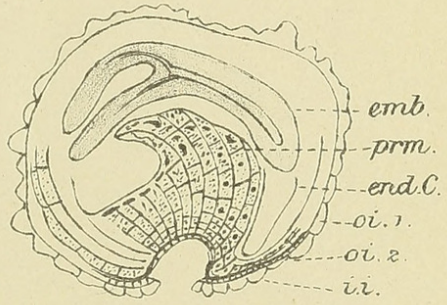
13.

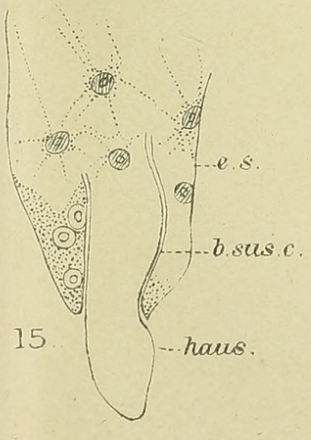


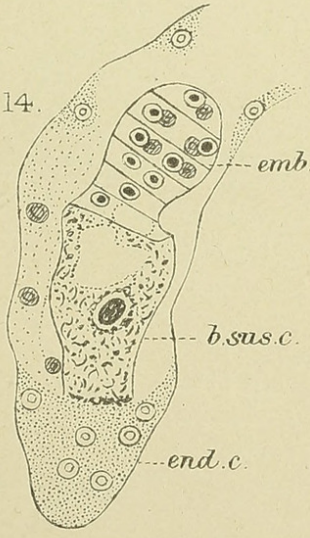


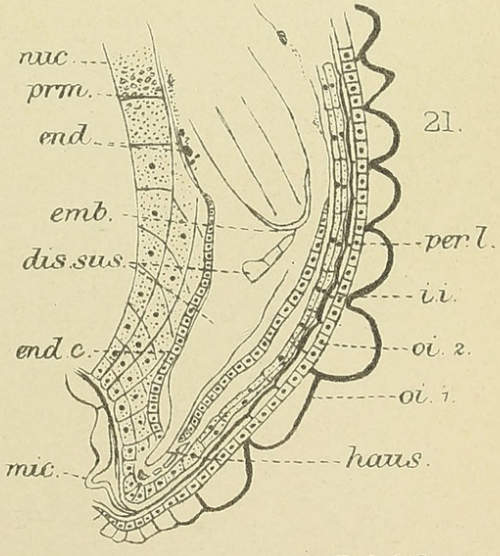




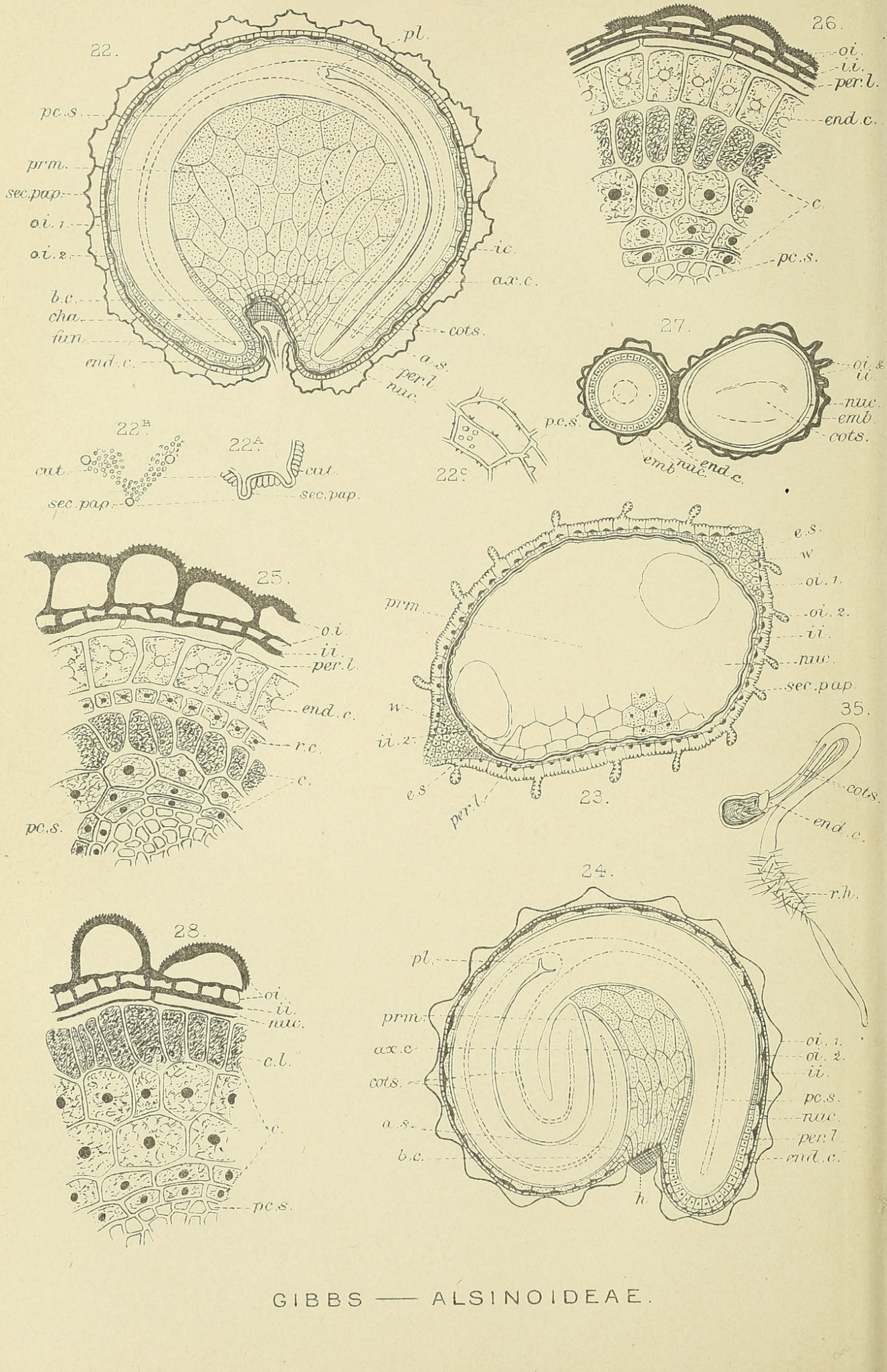


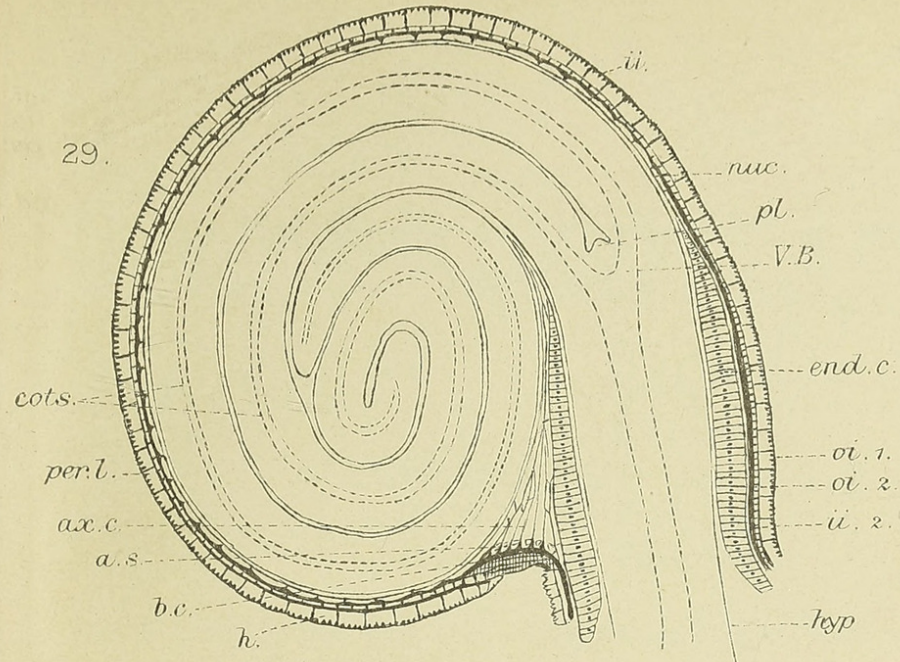


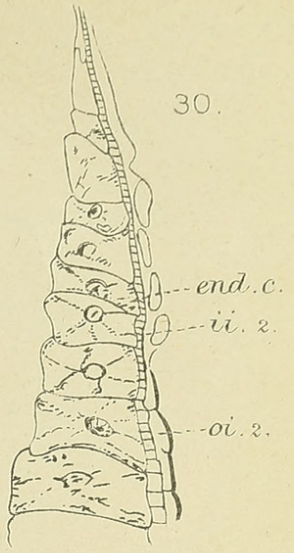


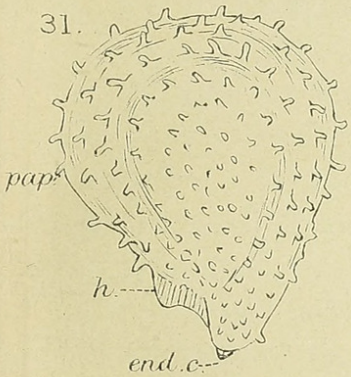


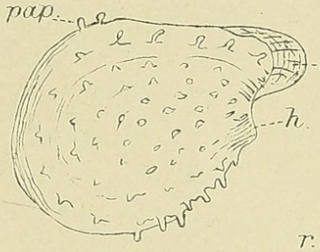
Huth, Lith. et imp.

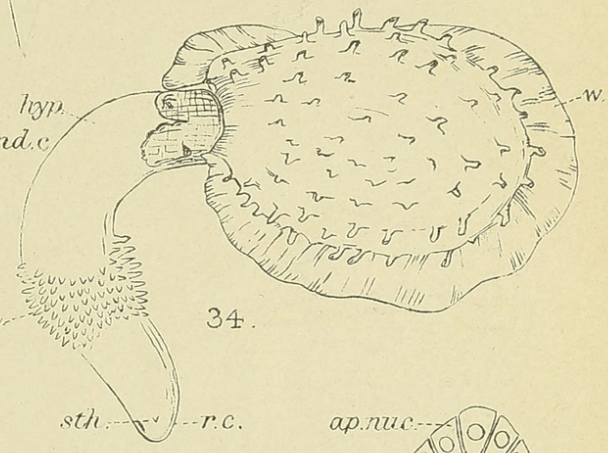

22.
pl.
pc.s.
prm.
sec.pap.
o.i.1.
o.i.2.
ic.
ax.c.
b.c.
cha.
fun.
cots.
end.c.
a.s.
per.l.
nuc.
22B
cut.
sec.pap.
22A
cut.
sec.pap.
22C
26.
oi.
ii.
per.l.
end.c.
c.
pc.s.
27.
oi. & ii.
nuc.
emb.
cots.
pc.s.
h.
emb.
nuc.
end.c.
25.
o.i.
ii.
per.l.
end.c.
r.c.
c.
pc.s.
e.s.
w
oi.1.
oi.2.
ii.
nuc.
sec.pap.
prm.
w
ii.2.
e.s.
per.l.
23.
35.
cots.
end.c.
r.h.
24.
pl.
prm.
ax.c.
cots.
a.s.
b.c.
oi.1.
oi.2.
ii.
pc.s.
nuc.
per.l
end.c.
h.
28.
oi.
ii.
nuc.
c.l.
c.
pc.s.

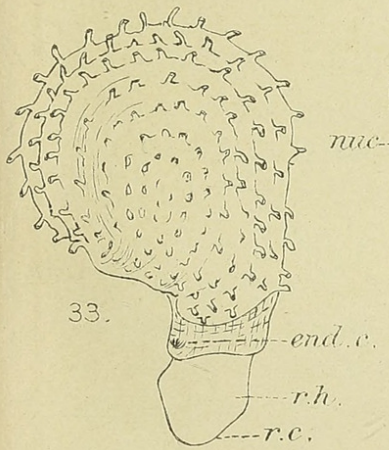


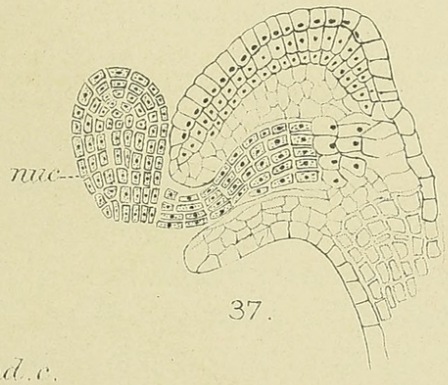


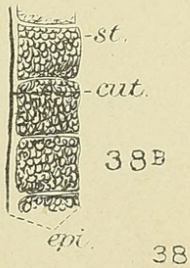


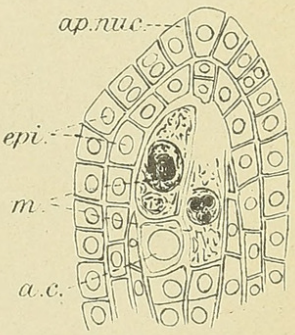


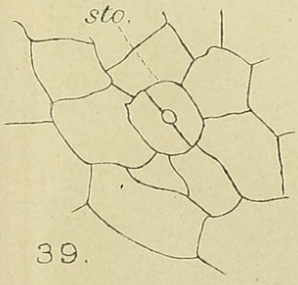


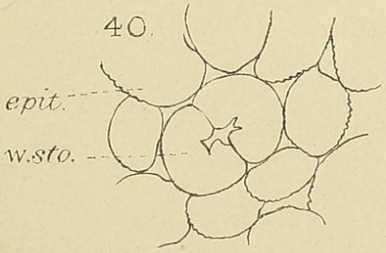


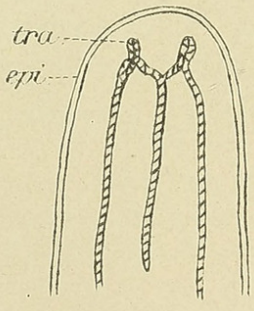


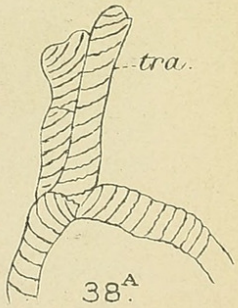












Huth, lith. et imp.