On the development of the Aleurone-grains in the Lupin.

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With Plate X. B.

THE formation of aleurone-grains, the characteristic proteid reserve-material found in seeds, was studied by Pfeffer[1] sixteen years ago. According to his results, the mineral contents, crystals of calcium oxalate, or the 'globoids' of double phosphate of lime and magnesia, first make their appearance in the cell-sap, and then, singly or in groups, act as centres of attraction for the proteid matter, which, as the seed in ripening loses water, is precipitated from the turbid cell-sap. Where proteid crystalloids occur, they too appear in the cell-sap simultaneously with the inorganic solids.

In describing their development in Lupin (referring more especially to *L. polyphyllus*), Pfeffer says, 'The protoplasmic strands having been converted into ground-substance, the resulting arrangement might at first sight easily suggest the idea that the protoplasm becomes a parenchymatous network whose meshes form moulds for the immigrating metaplasmic substance. But the history of development is opposed to such a conclusion.'

It would appear, however, at any rate in *Lupinus digitatus* which has been investigated in the present instance, that this

[1] Pringsheim's Jahrb. fur Wissenschaft. Bot. Bd. 8. 1872.

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rejected idea is more nearly correct than the conclusion at which Pfeffer eventually arrives, inasmuch as the grains are evidently actually secreted by and in the protoplasm itself.

Until the cotyledons completely fill the seed-coat, there is no trace of the aleurone-grains; the cells contain a conspicuous nucleus slung in the centre by thick protoplasmic bridles or sometimes lying in the parietal protoplasm. In the latter is the layer of chlorophyll-corpuscles in which small grains of starch appear, which, by gradual increase in size and number, have filled the corpuscles by the time the cotyledons have filled the seed-coat. When this stage is reached the seed begins to swell and its outline can be traced through the pod. If we examine sections of the cotyledons at this stage, the cells are seen to contain small spherical or oval bodies partly or wholly projecting from the granular protoplasm, whether the parietal layer, or that surrounding the nucleus or forming the connecting bridles (Fig. 1). These bodies at first appear as little convex protrusions, but rapidly increase in size till spherical or oval bodies are formed more or less embedded in the protoplasm. They stain deeply, more so than the protoplasm itself, with iodine, haematoxylin, Hofmann's blue, and eosin, and the staining is perfectly homogeneous. Nowhere in the cell is there any suspicion of solid mineral matter; crystals of calcium oxalate and globoids are alike absent.

If a section be mounted in iodine and watched while dilute potash (1 per cent. or 5 per cent. solutions were used) is run under the cover-slip, the bodies are seen to swell up considerably, and project into the vacuole, while the substance contained in them evidently dissolves. In the now very transparent section their fine clear distended outlines are seen to be in continuity with the protoplasm. If we now carefully wash, by drawing a little water through, and then run in iodine, the section shrinks and again becomes stained, but the deeply staining bodies have gone; we can still see however, especially in the uncompressed cells towards the outside, the delicate stained protoplasmic membranes in perfect

continuity with the rest of the protoplasm and enclosing the cavities from which the soluble matter has been abstracted (Fig. 2). It is therefore evident that the above-mentioned bodies consist of some substance, presumably proteid, soluble in dilute potash, which has been secreted by and in the protoplasm.

If sections be similarly treated with 10 per cent. or saturated solutions of common salt or potassium phosphate, the bodies merely swell up somewhat but are not dissolved, and, if washed in water, even after lying for twenty hours in the salt solutions, appear quite unaltered. 1 per cent. and 10 per cent. solutions of hydrochloric acid, even after twenty hours' action, only cause slight swelling. The bodies therefore differ in solubility from the grains of the ripe seed, which are completely and at once soluble in such solutions.

After solution a perfectly clear space is seen to remain, and there is no sign whatever of crystalline or globoid contents.

These bodies, which, as the sequel shows, are the primitive aleurone-grains, increase in size and number and soon fill up the vacuole, so that the cell contains within the parietal layer of protoplasm a number of roundish grains quite separated from each other by a protoplasmic reticulum, made up of the bridles and the membranes originally separating the secretion from the vacuole. By watching a section in which this stage has not quite been reached, while dilute potash is run under the slip, the limiting protoplasmic membranes of adjacent or opposite masses of the secretion are seen to swell out and meet to form what has now every appearance of a protoplasmic strand, indicating how the same would take place in the ordinary process of growth. Near the centre, or sometimes at the side, is seen the nucleus, which is becoming more or less compressed by the growing grains; these relations are clearly brought out by iodine, and the protoplasmic network demonstrated by running in dilute potash which at once dissolves the grains, leaving quite empty cavities.

By the time the vacuole has been nearly filled up, a dif-

ference in solubility is noticed, the grains now reacting like those of ripe seeds, dissolving completely in 10 per cent. and saturated solutions of common salt and potassium phosphate, and also in 1 per cent. of hydrochloric acid, though still insoluble in water. One gets sometimes preparations in an intermediate state with the grains only partly soluble, even after twenty hours' exposure to the reagent. It has been shown[1] that the aleurone-grains of ripe seeds contain several distinct proteids belonging to the albumose and globulin groups, and the change in solubility during development may be the expression of the breaking down of some complex proteid substance, originally secreted by the protoplasm, into the several simpler proteids known to occur in the ripe seed, and it is during this process that one would expect the separation of solid mineral constituents to take place in cases where they are found in the ripe seed. The grains continue to increase in size but are at first rather watery, and in absolute alcohol material show a vacuolation, probably due to the reagent, the denser part forming an external ring, or very often collecting chiefly on one side and forming a crescent (Fig. 4); the ring or crescent stains well with the above-mentioned dyes, while the portion inside remains clear. On solution, however, the denser portion is seen gradually to diffuse throughout the whole, forming a homogeneous structure (Fig. 5); when this stage is reached the seed is beginning to get ripe, as indicated by the end of the radicle turning yellow. As ripening goes on the denser part encroaches more and more on the clearer, and by the time the yellow coloration has extended up the radicle and is affecting the cotyledons, the majority of the grains have again come to stain homogeneously, as in the ripe seed, indicating increase in quantity of the denser part and loss of water of the grain coincident with the general drying of the seed. The protoplasm has meanwhile been diminishing, and the starch-grains have by the end of this process disappeared, drops of oil

[1] Vines, Journal of Physiology, III, 1881.

having however been formed. In the ripe seed the grains, which are roundish or somewhat angular through mutual compression, are still separated by a protoplasmic network in which oil-drops occur, while starch is wanting. Hanstein's solution brings out the network and nucleus very well, staining these a deep violet, while the grains scarcely stain at all (Fig. 7).

Solid inorganic constituents were repeatedly sought for, but without success. Sections of the ripe seed, from which the oil had been removed by ether, were treated on a slide with 1 per cent. of potash, which was allowed to diffuse in so as not to wash away any small globoids which might be present; individual cells or grains were carefully watched meanwhile, sometimes under Zeiss' F objective, at others under the D, but in all cases an empty space was left in the protoplasmic network after solution. Some granules scattered over the section, but especially, and almost exclusively, near the few cell-layers with very granular contents beneath the epidermis, and with no definite relation to the grains, proved to be small starch-grains washed out from these cells. No crystals could be detected by double refraction when such a section was examined under a polarising microscope. Hence we may conclude that the aleurone-grains of *Lupinus digitatus* have no solid mineral contents. From the foregoing facts it appears that the presence of mineral matter is of very secondary importance in the development of the grains, whereas in the process as described by Pfeffer the mineral matter was essential, forming the point of attraction for the aggregation of the proteid. But Pfeffer's suggestion is too mechanical, and moreover gives no reason whatever for the fact that the grains in the ripe seed are always embedded in a protoplasmic matrix; they should rather be lying loose in the vacuole.

The earliest stage, namely, secretion in the protoplasm of matter soluble only in dilute potash, has also been observed to occur in precisely the same way as above described in another species of lupin (?*L. varius*).

It is most interesting to note that the development of

aleurone-grains described here corresponds most closely with the manner of secretion of mucilage as lately described [1] by Gardiner and Ito in the glandular hairs of *Blechnum* and *Osmunda*; in both cases the secretion is strictly intraprotoplasmic, both the aleurone-grains and mucilage-drops moreover remaining, after secretion, quite separate in a reticulum of protoplasm. In both cases too there is some chemical change in the originally secreted substance, before the final product is formed.

The seeds used in these investigations were preserved in absolute alcohol; 2 per cent. chromic acid material shows the early stages very well, but as the grains begin to increase in size, the cells are seen to be full of empty rings, an appearance which is maintained up to the time when the seed is fully ripe; the grains are moreover rendered quite insoluble, even in the ripe seed, in salt solutions and 5 per cent. potash. By placing sections of the ripe seeds, preserved in alcohol, in 2 per cent. of chromic acid solution, the homogeneous grains are converted into rings, which now resist for several minutes the action of 5 per cent. potash and remain undissolved, even after twenty hours, in saturated salt solution.

The development of aleurone-grains in general is obviously not completely indicated above, as no account is taken of the time and manner of appearance of the globoid and crystalloid, which may both be present, as e. g. in *Ricinus communis*, though *Lupinus digitatus* has neither. I hope to work out these points also, in the summer, when material can be procured.

To judge from the title [2], which alone I have seen, and that only a few days since, my results agree with those arrived at in a paper by Wakker.

[1] Annals of Botany, I. 1. 1887.

[2] 'Aleuronkorrels zijn vacuolen,' in Maandblad voor Natuurwetenschappen, Nos. 5 and 6, 1887.

EXPLANATION OF FIGURES IN PLATE X. B.

Illustrating Mr. Rendle's paper on the development of Aleurone-grains in the Lupin.

Fig. 1. First stage in formation of aleurone-grains in *Lupinus digitatus*. Drawn from a preparation stained with Hofmann's blue. Portion *m*, unshaded, not in focus. The bodies mentioned in the text are line-shaded. Zeiss' D objective and ocular 4. *n*, the nucleus.

Fig. 2. Same stage as the last, showing the little pockets in the protoplasm from which the secretion has been dissolved out by dilute KOH. Now in iodine. The swollen starch-grains shaded dark. *a* and *b* same magnification as in Fig. 1. *c* under F objective. *n*, the nucleus.

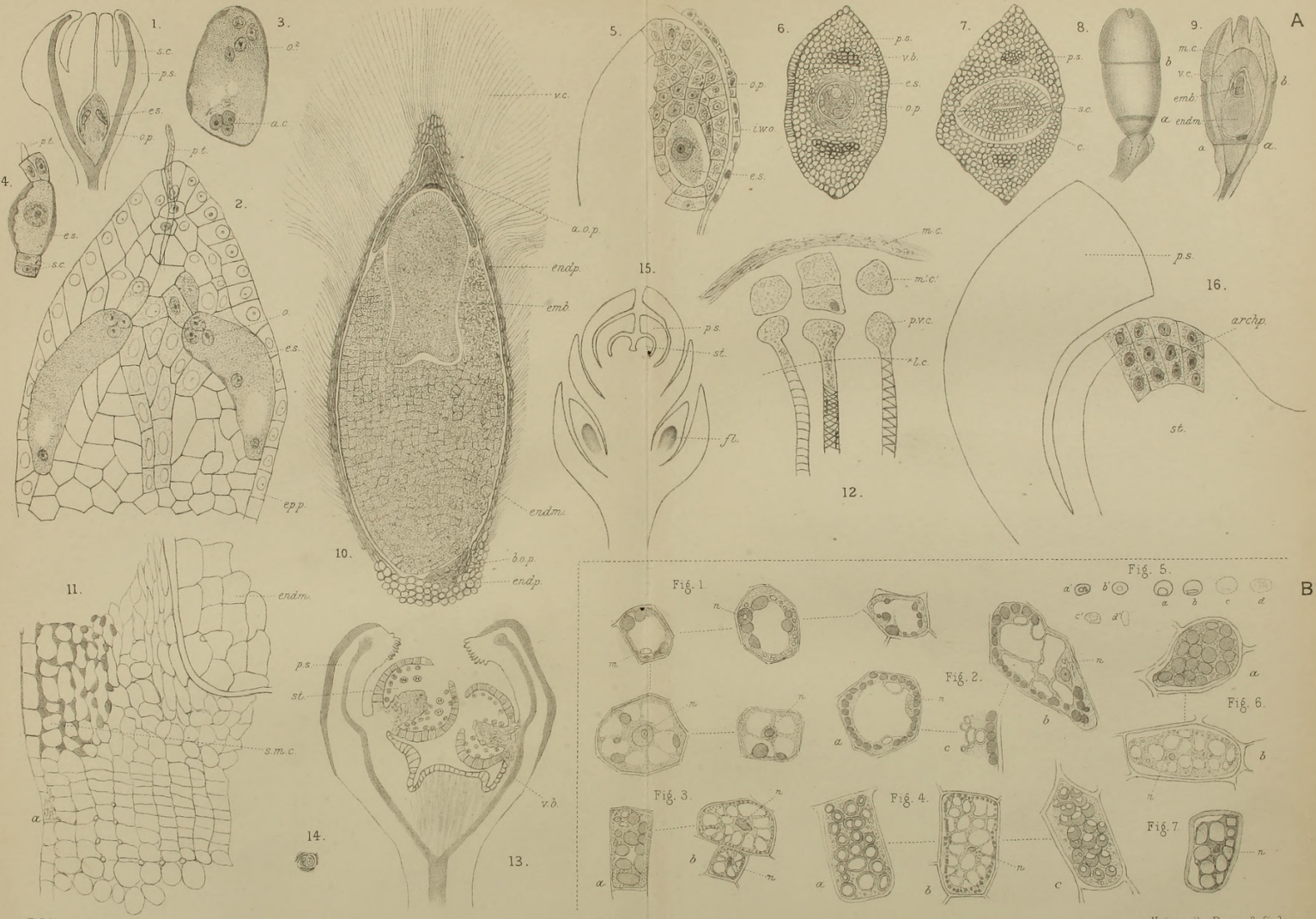
Fig. 3. A little older than the above, grains filling up the vacuole. *a* and *b* both from preparations stained with Hofmann's blue; *b*, after action of dilute KOH showing the protoplasmic network. D objective, ocular 4. Colourless starch-grains seen in the protoplasm lining the wall.

Fig. 4. The growing grains largely fill the cell. In it are seen the grains vacuolated as described in the text, and colourless starch-grains in protoplasm, after staining with Hofmann's blue. *b*, after solution of grains with dilute KOH, and staining of protoplasmic network with iodine. Swollen starch-grains lining the wall. *n*, the nucleus. D objective, ocular 4. *c* a little older than it.

Fig. 5. Shows progress of solution of two vacuolated grains, in dilute KOH on the left, in 10 per cent. of salt solution on the right. D objective, ocular 4.

Fig. 6. From a nearly ripe seed. *a*, a cell before, *b*, one after action of dilute KOH. *a* shows the deeply and homogeneously stained grains, *b* the protoplasmic matrix and nucleus, *n*. The colourless drops are oil. D objective, ocular 4.

Fig. 7. From a quite ripe seed stained with Hanstein's solution. Nucleus and protoplasmic matrix have stained a deep violet, the wall a lighter colour, the grains almost perfectly colourless. *n*, nucleus. D objective, ocular 2.



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