

Sorghum vulgare.—The aleurone-layer of the seed consists of a single layer of flattened cells. In them there is protoplasm, oil, minute starch-grains, and aleurone-grains which, however, have *no globoids*. No crystals are formed in these cells when they have been treated with ammoniacal ammoniac chloride and hydrodisodic phosphate. In the cells of the embryo occur fat, much starch, and aleurone-grains with large globoids. The distribution of the starch-grains corresponds with that of the globoids. Hence in this seed iodine-reagents have to be employed after the usual tests for globoids have been performed.

Zea Mays.—The aleurone-layer is single. In it the aleurone-grains contain small globoids, but have a larger proportion of proteids than in any of the preceding types in which globoids are present. When sections are placed in ammoniacal ammoniac chloride and hydrodisodic phosphate, a small quantity of crystals form in the aleurone-layer. If placed in sulphuric acid, crystals of calcic sulphate form in these cells. After treatment with alcohol the proteids are insoluble in dilute potassic hydrate (1 per cent.). The embryo contains oil, and aleurone-grains with globoids.

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ON NUCLEAR DIVISION IN THE POLLEN-MOTHER-CELLS OF LILIUM MARTAGON.—Having been for some time past engaged in researches upon the mutual relations of the cytoplasm and nucleus, during spore-formation, in certain of the lower plants, I became desirous of working through some well-known type of division in the corresponding cells of a phanerogam, in the hope that some of the difficulties which presented themselves, during the investigations referred to, might thereby be explained.

For this purpose I selected the anthers of *Lilium Martagon*, in which the changes which accompany the development of the pollen grains had already been studied by Guignard¹. He found, and my experience also confirms this, that alcohol is on the whole the best fixing and preserving medium for plant-cells, and my observations soon became chiefly directed to material which had thus been fixed.

I made use extensively of the various and numerous stains commonly associated with researches of this nature, both with and without the additional employment of mordants, but after a large number of

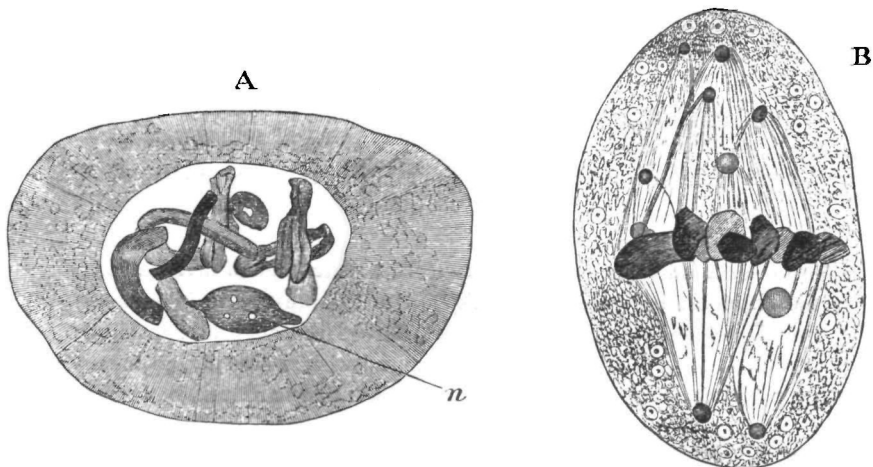
¹ L. Guignard, *Nouvelles études sur la Fécondation*, An. sci. nat. Bot., sér. 7, t. 14, 1891.

experiments, I came chiefly to rely on safranin, haematoxylin, fuchsin, methyl-green, gentian-violet and orange-G. On the whole, the best results were obtained by slow staining in haematoxylin, followed, after washing out the surplus stain, by further treatment with a watery solution of orange. Both gentian-violet and safranin, especially when used in conjunction with orange, gave good results. The sections thus prepared were mounted in glycerine, or glycerine and chloral hydrate, or were cleared and mounted in Canada balsam, and for double or triple stained preparations I much prefer the last mentioned mounting-medium. Extremely satisfactory results were obtained, in the case of the haematoxylin-orange stain, by treating the sections stained with haematoxylin, after careful washing, with a weak solution of lead acetate, and then finally, after again washing, staining with orange. Zinc sulphate was also tried in the same way, but was found not to give such good results. All the above methods led to the same conclusions, though with various degrees of clearness, and as my observations are somewhat out of accord with those commonly accepted, I thought it best to communicate those results which appeared to be of the greatest interest, reserving a detailed account for a future occasion.

On examining the pollen-mother-cells at that stage of division when the chromosomes (which, as Guignard stated, are twelve in number) are aggregated in the equatorial plane, and the achromatic spindle is well defined in the cytoplasm, I found what I was by no means prepared for, namely, that in the cytoplasm there are scattered about a number of *granules*, which were not figured in the plates accompanying the memoir already referred to; and that these granules are coloured by those stains which differentiate the chromatic elements of the nucleus, and are thus very clearly defined in the cell-protoplasm. They occur for the most part, though by no means entirely, in the region of the achromatic spindle, and the point of interest connected with them is this, that many of them are obviously related to the spindle-fibres, and mark the position of attraction-centres for parts of the spindle which is thus *broken up and becomes multipolar*, if one may use such an expression. This character is illustrated by the figure (Woodcut 2, B), which is intended to reproduce an actual *camera lucida* drawing. The granules which are thus related to the spindle-fibres are very variable in number, and are equally so in position; a number of granules, however, so far as I have observed,

always remain neutral. Those, however, which are so related to the fibres, exert a most obvious influence on the direction of these, and a whole sheaf or strand of spindle-fibres may sometimes be seen to divert from the main direction, and to end blindly on such granules.

I have been unable to demonstrate the existence of special centrosomes occupying definite positions at each end of the cell, and I am not clear as to how they might fit in with the granules. Perhaps it is conceivable that in some cases the individual granules might fuse and form one large terminal body which one might then regard as a 'centrosome,' and it is certain that in many cells the main portion of the



Woodcut 2—Fig. A. Pollen-mother cell of *Lilium Martagon* in early stage of division: *n* the nucleolus. B. Later stage, showing multipolar spindle.

achromatic spindle does stand in relation to bodies near the poles of the cell, but even in these cases there are almost always to be seen, in preparations which allow of a definite conclusion being arrived at, divergent strands separating from the main spindle and terminating in granules otherwise situated. Eventually, during the separation of the daughter-chromosomes, the spindle seems to become more regular, but the difficulties of tracing its real relationships simultaneously increase, and I do not as yet feel able to speak with entire confidence as to its further fate.

Shortly after I had, somewhat unwillingly, arrived at the conclusion

that these granules, with their curious relations to the spindle-fibres, were regular and normal constituents of the cell during these stages of division, I learnt with pleasure from my friend, Mr. J. E. S. Moore, that in the course of his investigations into the behaviour of dividing cells in animals, he had come across a similar case of multipolar spindles in *Branchippus*. His results are in course of publication, but he kindly invited me to see his preparations, and they agreed with those of *Lilium* in their essential characters so far as the relations of the spindle are concerned. Of course multipolar spindles in plants are not new, but those hitherto described¹ are of a different nature from those which occur in the Lily anthers. Moreover they are found in endosperm cells, whose nuclear constitution appears to vary within considerable limits, at least judging from the existing accounts before us. In such a cell-division as that which results in the production of a spore, it seems, however, possible that their occurrence may be of some special significance, but I reserve the discussion of this point for a future occasion.

Hitherto I have only described the cells when in a state of active division, but when these stages are compared with earlier ones, several other features of interest become prominent. The cytoplasm up to the time immediately preceding the disappearance of the nuclear membrane, and the aggregation of the chromosomes in the equatorial plane, is *perfectly free from stainable granules*. The nucleus, when preparing for division, exhibits a much convoluted thread with rows of dots, which stain deeply, running along the edges, exactly as is beautifully shown in Fig. 10, accompanying Guignard's memoir already referred to. The nucleolus is of enormous size, and contains usually several endonucleoli. At a stage somewhat later, when the chromosomes have become individualized, and are lying irregularly disposed within the nucleus, the nucleolus almost always assumes a most curious and characteristic shape, that of an oval body with two polar protuberances (Fig. 4). Frequently it lies close to the cytoplasm, one of its pointed ends actually appears to jut out into it; sometimes, however, instead of one large nucleolus, stages in its fragmentation may be observed, and a number of smaller bodies, which present precisely the same staining-capacity take its place. Their aggregate size, however, is equivalent to that of the large single nucleolus.

¹ Cf. Strasburger, *Histologische Beiträge*, Heft 1, 1888, especially Taf. III, Fig. 34, and the text.

Immediately following this stage in the nuclear division, the chromosomes congregate in the equatorial plane, and simultaneously the granules already referred to in the earlier part of this communication are discoverable in the surrounding cytoplasm. They stain precisely as do the granules which arise in the nucleus by fragmentation of the nucleolus, and are very distinctly seen, many of them influencing the direction of the spindle-fibres as already described. It would at present be premature to attempt to do more than suggest that there may be a closer connexion between the granules of nucleolar origin and those which later occur in the cytoplasm, but it may be mentioned that Hertwig¹ suggested the possibility of a nuclear origin for the animal centrosomes. There are certainly great difficulties in the way of accounting for the sudden and abundant formation of granules on the assumption of a purely cytoplasmic origin, and their obvious relation to the spindle-fibres, as well as their ultimate fate, opens out a whole set of further questions. I will not advert to them at greater length here; I hope to be shortly able to speak more definitely on these, as well as other points raised in this preliminary note.

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THE GENUS TREMATOCARPUS².—At Mr. Hemsley's request I have examined the structure of the fruit of *Lobelia macrostachys*. Dr. Zahlbruckner's note on his *Trematocarpus macrostachys* may be divided into two portions, the first of which is decisive for the second, viz. the structure of the fruit, particularly with regard to dissemination, and the generic value of the characters derived from that particular structure. The remarks which I have to make on this structure are based upon observations on the material preserved in the Kew Herbarium, and on a fruit sent by Dr. Zahlbruckner to Mr. Hemsley.

The ovary wall of *Lobelia macrostachys*, Hook. et Arn., exhibits the same general structure as in allied species of *Lobelia*, the generic identity of which has never been doubted. One remarkable character is the presence of a well-developed system of vascular bundles anastomosing in a distinct network very like that found, for instance, in *L. nicotianaefolia*, an Indian species, with the only difference that it is

¹ O. Hertwig, *Die Zelle und die Gewebe*, Jena, 1892, p. 48 and 164.

² See *Annals of Botany*, vol. vii. No. 26, and vol. vi. No. 21.