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V. On the Staining of Microscopical Preparations

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16. *Leucodontaceæ*—
Antitrichia curtipendula.
17. *Hypnaceæ*—
Plagiothecium undulatum.
denticulatum.
sylvaticum. (In a ravine on Ben Each).
Muhlenbeckii.
Thamnium alopecurum.
Eurhynchium striatum.
prælongum.
Swartzii. (Quartz rocks on Ben Each).
crassinervium.
Hyocomium flagellare.
Isothecium myurum.
myosuroides.
Brachythecium salebrosum. (A pale coloured form, with
bright-red smooth capsules).
Limnobia ochraceum.
var. fluitans.
Hypnum cupressiforme.
molluscum.
crista-castrensis.
revolvens.
sarmentosum. (In fruit in Corrie Glass).
commutatum.
scorpioides.
Schreberi.
Hylocomium brevirostrum.
umbratum.
triquetrum.
splendens.
squarrosum.
loreum.
Oakesii. (Rocks at Loch Maree).

V. *On the Staining of Microscopical Preparations.* By Dr
W. R. M'NAB.

1. *Staining with Acetate of Mauvine.*

A. This can be used with very good effect in staining thin sections of wood. If these are preserved in Canada balsam they seem to be permanently stained, as a few specimens put up in January 1866 are now quite as brightly

coloured as they were at first. By means of the colouring the high powers of the microscope can be used, bringing out points of structure not easily demonstrated without being so treated. Sections (transverse) of coniferous woods show beautifully the structure of the punctated tissue, as well as the junctions of the cell walls and the thickening layers of ligneous matter.

B. Sections of the young stems of ivy were last year (February 1868) successfully stained with acetate of mauvine and glycerine (see "Botanical Society Transactions.") Since then most of the specimens have faded. The contents of the cells have lost colour entirely, but the cuticular layer of the epidermis retains its colour, as well as the young ligneous cells. In a specimen now before us, the pith and the enclosed cell-contents are now colourless, but the zone of cells in which a deposit of ligneous matter has been formed are brightly coloured. The very small cambium cells bounding this layer are quite colourless. In the outer layer of cambium the cells become large, more like the bark cells, and here and there a few bundles of thickened cells can be seen, brightly coloured like the others. These, I suppose, are *bast cells*. A few of the laticiferous canals, described last year in "Botanical Society Transactions," are lying close to these small patches of bast-cells, but far more frequently they lie close to the small cambium cells. The layer of cellular tissue, external to the bast cells, is not coloured, and their thin walls contrast strongly with the greatly thickened cell-walls of the epidermal cells, on which the mauvine does not seem to exert any influence. The cuticle is well marked as a bright purple external layer, contrasting strongly with the colourless thickened cells on which it rests.

If any reliance is to be placed in the results obtained by staining specimens with mauvine, there is one very important point I wish to mention. Schacht* states that the so-called intercellular substance, joining the walls of contiguous cells together, forms on the free surface of cells what is known as the cuticle. By some the existence of this intercellular substance is denied; and by careful examination of many specimens now before me, I can find no

* Grund. der Anat. und Phys. der Gewächse, page 29.

trace of any such material. The cuticle is brightly coloured, and if the same material existed as intercellular substance between the cells, a coloured layer should be seen between each of these cells, but none can be seen. If, then, the test of colour can be relied on, either intercellular substance does not exist, or else its composition is essentially different from that of cuticle.

2. *Staining with Beale's Carmine Solution.*

When small portions of vegetable tissue are soaked in the carmine solution, only those cells containing protoplasm appear stained. The nuclei and the granules in the protoplasm seem alone to be affected. The depth of colouring depends, then, on the number of granules in the protoplasm and the size of the nucleus.

A. *Experiments in Phalaris canariensis.*—The rootlets of germinating seedlings of *Phalaris canariensis* were placed in a solution of carmine. When examined about twelve hours afterwards, the extremities of the rootlets were found to be very deeply coloured, while a faint tinge was visible along the other parts of the rootlet. Under the microscope the structure of the growing point of the root was beautifully seen. First a series of loose cells are seen at the extremity, continuing a short distance above the growing point. A very few of the most internal cells seem to be slightly coloured, the external ones being quite colourless. This mass of cells forms a regular sheath-like cap to the growing part of the root, very similar to the highly developed pileorhiza of *Lemna*. A well marked line of separation can be seen marking the boundary of the growing point, the cells being deeply coloured for a short distance. The cells are densely filled with granular protoplasm, and each contains a very large nucleus. A little higher up the colouring gets very faint, and at last is confined to the interior of the rootlet, where a few nuclei are seen closely surrounding the spiral vessels. The growing point of a root of the *Phalaris*, when treated with carmine, shows—1st, A number of large loose cells at the point, extending like a pileorhiza, a short distance from the extremity; 2d, The internal cells of this sheath are coloured, and therefore contain active protoplasm; 3d, The growing point of the root is very

deeply coloured, the protoplasm is densely granular, and contains a large nucleus; 4th, A very short distance above the growing part the cells are only marked by an occasional nucleus; and, lastly, the nuclei are only found close to the spiral vessels.

B. *Experiments with Sinapis alba (White Mustard)*—*Series 1.*—These were begun in February 1868, and consisted in germinating white mustard both in water and in soil, and then examining the structure after staining with carmine. During the first stages of germination of the seeds, germinated both in soil and in water, no trace of staining could be found, and it was not till the radicle had attained some size that the point of the root became stained with the carmine. If we can depend on such tests, we must conclude that the germination of the white mustard is not dependent on the formation of *new cells*, but merely on the growth and enlargement of the pre-existing cells. Whenever growth by the formation of new cells had taken place these cells could be rendered distinctly visible by the action of carmine, the parts above this remaining white, although submitted to the action of carmine under precisely the same conditions.

Series 2.—A much more extensive series of experiments was tried in December 1868, by growing white mustard for a short time in carmine solution. After the plants were about two inches in height carmine was added, and they continued to grow for several days after. The youngest stages of development failed to furnish stained specimens, and it was only after a few days that the young radicles could be stained. The structure of the root is more complex than that seen in the *Phalaris canariensis*, but agreed in every essential point. The loose cells were seen at the tip, the growing point densely coloured, the colouring becoming less and less till it was no longer visible. The protoplasm near the growing point is densely granular, and the nuclei very large, filling up from one-fourth to one-third of the whole cell.

Transverse sections of the young root, above the growing point, showed the arrangement of the cells very clearly. In the centre is a mass of small thickened cells, containing protoplasm and nuclei in many cases. Immediately outside this are three layers of large cells with protoplasm and

large nuclei. Surrounding this layer is a single series of small cells placed very close together, containing a densely granular protoplasm, and very large, often two, nuclei, probably a cambium layer. External to this is a layer of epidermal cells, not apparently thickened, the whole being enclosed by what appears to be a thickened cuticle. Parts of the young radicle are covered with hairs. These could be very clearly seen, and their mode of formation observed. I cannot detect any thickened cuticle at those parts where the hairs are developing. The hairs are direct prolongations of the epidermal cells, and in general the contents and nucleus can be seen to have been carried forward, and occupying the centre of the hair. The sections I have now before me, showing the hairs, are much farther from the growing point than that described above as having a thickened cuticle, but no cuticle is visible. The hairs elongate very much, without dividing; the nucleus in most was wanting, although the granular contents remained. Here and there large spaces filled with cell-sap were seen, and in one instance the contents of one of these long cells were seen oscillating backwards and forwards for some time. The use of the staining process does not seem to be attended with any great difficulty, and, I have no doubt, important results may be obtained by careful study of its action on germinating plants.

VI. *Letter from Dr R. O. CUNNINGHAM to Professor BALFOUR, dated H.M.S. "Nassau," Valparaiso, 3d November 1868.*

Rather more than a year ago I wrote to you, describing our experience during the first season we spent in the Strait of Magalhaens, and possibly you may be interested by a few notes of our proceedings during the past year. I shall confine myself principally, in the following remarks, to what I have observed in the way of botany:—

We entered the strait on the 17th of November 1867, and reached Sandy Point two days later, on a lovely spring morning, recalling the month of April at home. The fresh green foliage of *Fagus antarctica* was very refreshing to the eye after our sea cruise, and a considerable number of