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THE IMPORTANCE OF STRUCTURAL DETAILS IN THE STUDY OF PLANTS.

BY W. W. ROWLEE, ITHACA, N. Y.

If there was any one thing more than another that led the ancient herbalists into serious and often amusing errors in regard to plants, it was their ignorance of the structure of vegetable tissues. Their minds were not in a condition to be attracted by natural phenomena, and, further, they were without lenses and other appliances which are so indispensable as aids to workers of the present day.

It is, moreover, but recently that some important advances have been made in methods of treating tissues in order that their structure may be more thoroughly and exactly studied. With the exception of those which are lignified or sclerenchymatous, all vegetable tissues are too soft and yielding to be cut even with the sharpest knife without undergoing displacement and distortion, and especially is this true of that part of the tissue most important of all from a physiological point of view, i. e., the protoplasm. In its normal condition in the vegetable cell, protoplasm resembles in texture and consistency the white of an egg. No one would expect to cut this substance in its natural condition into sections to be examined with a microscope. The cell-walls also are displaced when unfixed tissue is cut, especially if the tissue be from delicate organs. If fresh material could be cut and examined without any distortion it would be highly desirable to do so, as all parts would be unchanged, but not only is this impracticable on account of the firmness of the tissue, but, moreover, certain parts are in nature colorless and must be stained before they can be seen.

Fixation of tissues consists in hardening and preserving the pliable and perishable parts of tissues, especially protoplasm and its primary modifications. This is accomplished by replacing the water in the tissue by some preserving fluid. The tissue must be killed immediately,

and the elements must remain *in situ* through the process of infiltration if the fixing is properly done. Professor Gage in his "Histology" recommends picric-alcohol (25 p. c. alcohol + .2 p. c. picric acid) for fixing tissues, and it has given us entire satisfaction so far as fixing material is concerned. The stain made by picric acid, however, is not altogether satisfactory. Professor Campbell (*Bot. Gaz.* Feb., 1891) found chromic acid very effective as a fixing agent in his work upon the delicate tissues of fern prothallia. We have been satisfied with results attained from using alcohol, always being careful at first to apply only sufficient to kill the tissue and then gradually to increase the strength of alcohol until the tissue is completely dehydrated. The most satisfactory method of accomplishing this we have found is by the modification of Schultze's apparatus devised and described by Professor Thomas (*Proc. Am. Micro. Soc.*, 1890). If the fixing has been properly done all the tissue elements will be *in situ* and normal except in so far as the alcohol produces changes. The tissue after fixing has been accomplished instead of being flexible is exceedingly brittle and must during succeeding manipulations be handled with the greatest care. As a general rule it is safer to pour pieces of delicate tissue from one vessel to another rather than to handle them with forceps. In this condition, protoplasm and cell-walls are firm enough to withstand the knife without displacement, providing proper support is afforded.

This support may be gotten by infiltrating the tissue with some liquid which under changed conditions will become solid. Here, too, the agents used should be such as modify the tissue as little as possible. Paraffin and collodion have been most used. Both will penetrate not only the intercellular spaces and cell cavities but will also infiltrate into the cell-walls and protoplasm, preventing the former from tearing and holding the nucleus and plastic bodies of the latter in position during sectioning. The effects of alcohol and other reagents upon tissues should be determined carefully by experiment, and such changes as occur should be considered before conclusions are drawn.

The importance of exactness in structural studies can scarcely be over-estimated. Confirmation or modification of new systems of classification (and there are plenty of them at the present time) must depend for their permanency quite as much upon accurate observation of structural details as upon the ingenuity of any systematist. A single illustration from a discovery made in our laboratory may not be inappropriate. The nodding bidens (*Bidens cernua*) differs from its congeners of the northeastern United States in having the hypocotyl of the embryo in the seed before germination possessed of large and numerous intercellular spaces. The occurrence of large intercellular spaces in an embryo is of comparatively rare occurrence and is probably of assistance to the plant in aerating its tissues during germination. This seems the more probable when one considers that the first structural modification attending germination is the enlargement of the intercellular spaces. In species in question the provision of intercellular spaces has been provided before germination. If this organ had been cut fresh the knife would have displaced the cells so as to render uncertain the relation of the cells and spaces.