

or foil, immediately above the sections, and allowed to flow down over them quickly. Very careful attention is now needed to control the action at the proper moment. In the course of three or four seconds the sections acquire a faint brownish color, which rapidly deepens as the dehydrating action of the acid proceeds. Its first appearance indicates, in most cases, that the action has been continued long enough. The slide is, therefore, quickly plunged in a dish of water which must be ready for that purpose, and the sections thoroughly washed. They are then ready for staining.

The action of the acid, dependent upon its dehydrating properties, is first to contract the protoplasm. It next causes the cell wall to swell strongly and partly dissolve, thus rendering it so transparent as to permit the threads of protoplasm which traverse it to be seen distinctly when stained. The swelling of the wall also tends to aid in the contraction of the protoplasm, while the channels become longer, and further aid in defining the filaments. If great care be not used in this process, the section will be quickly and wholly dissolved. This process is of special advantage as a quick method, while it gives most gratifying results, and it has been chiefly relied upon by us. It may be employed in ordinary parenchyma tissue, and also with great advantage in collenchyma and bast, to the treatment of which latter two, it is best adapted. This is one of the oldest of all the methods now in use.

The third method, and that which Gardiner seems to regard with the greatest favor is the chloriodide of zinc process. This admits of two variations; in the first, the sections are immersed for a short time in an ordinary aqueous preparation of iodine, until the characteristic reaction is developed. They are then transferred to the chloriodide, when they quickly turn dark brown, owing to the intensity of the iodine reaction. After about ten to thirty minutes in this latter reagent, they are washed out in distilled water until the brown color disappears. This method is said by Gardiner to have the special advantage of causing the protoplasm in all its parts, to take a much deeper stain when finally colored with aniline. The second variation simply omits the preliminary treatment with iodine. Preparations by this method, show the filaments very distinctly, and the walls of the cells so strongly swollen as to render them quite transparent. It may therefore be used instead of the last process by sulphuric acid.

Sections treated by any one of these methods, require subsequent staining, in order to differentiate the delicate filaments from the surrounding cell wall. The method originally employed by Tangl<sup>1</sup>, in the case of endosperm cells, was to stain with iodine. Our present methods, however, permit of much more accurate results. The stain recommended by Gardiner<sup>2</sup> as used by us, gives most satisfactory results. It is prepared as follows:—To a 50 p. c. solution of alcohol, add picric acid to saturation. To this add aniline blue (we used BB with good results) until the residual color imparted to a section, is deep blue. To facilitate solution, one or two drops of acetic acid may be added to the stain with advantage. Sections previously treated and well washed, are immersed in the stain for a few moments and then washed out in fresh alcohol until the yellow is all discharged and the color of the section changes from green to clear blue. It will then be found that the picric acid, in passing out from the section, has withdrawn all the aniline from the cell walls, but that it has left it in the protoplasm, for which it has a special affinity. The colorless cell walls

<sup>1</sup> Pringl's, Jahrb., 1880, 170.

<sup>2</sup> Phil. Trans., clxxiv. 817.