A MICROANALYSIS OF THE EPIDERMAL CELL WALLS BENEATH THE MIDRIB OF THE HOLLY LEAF

JOSEPH P. MCMENAMIN

Springfield Junior College, Springfield, Illinois

In stained sections of *Ilex opaca* leaves that had gone through the paraffin process two interesting features were observed. There were thick-walled guard cells that appeared to be "petrified" by lignification and dependent in functioning upon adjacent non-lignified epidermal cells, and there were lower epidermal cells on the midrib exhibiting a distinct absence of lignin in the outer tangential walls. A microanalytical study of these latter cell walls with differentiated lignification was made.

Sections of living, mature holly leaves collected in late January were cut through the midrib with a sliding microtome to thicknesses of 12 to 15 microns. Three types of tests were applied to these sections in determining cell wall constituents and their location. Different staining reactions were checked by differential solubility reactions and by the use of the polarized microscope. The deposition and the location of four wall constituents, cellulose, lignin, pectin, and cutin, were thus determined.

Chlorozinc iodide and the hydrocellulose reaction were employed for cellulose staining tests. Cuprammonia was used to dissolve cellulose, and since cellulose is an anisotropic substance the use of polarized light and interference colors served in checking the other tests. Both staining and solubility tests brought negative results until lignin was removed from the walls, after which, these tests gave distinctly positive reactions. The resultant swelling of the walls in staining tests made the pitting less evident. Cuprammonia dissolved the non-cutinized portions of the delignified walls. Sections left in cuprammonia to remove cellulose were of little value, there being no positive test possible in the first place. As a result of cutin saponification there were disclosed lamellated structures located in the inner cutin layer over each cell. These gave the positive blue and violet colors for the cellulose staining tests. They dissolved when cuprammonia was added to a slide and gave further evidence of their plated organization in breaking down. They were doubly refractive between crossed Nicols, but only to the extent where it was barely noticeable, whereas anisotropy of the rather thick secondary walls was actually strong. It seemed that the outer tangential

secondary wall was not quite as bright as the other secondary walls.

Two lignin staining tests were applied, the phloroglucin and the Mäule reaction, revealing a high concentration of lignin in all walls except the outer tangential, the cutinized wall. This wall seemed to be more pitted than the other walls, the irregular pits extending out to the cutin layer. Sections were delignified by soaking in Chlorox for three hours. Staining tests were then repeated to make certain all lignin had been removed, and negative reactions substantiated this treatment. Lignin staining tests applied to sections treated with cuprammonia showed no apparent differences, unless a more sharply defined reaction. Sections stained by chlorozinc iodide were observed to have a brownish color which is often indicative of cellulose "masked" by lignin. Optical properties were of little help here as lignin is amorphous and isotropic. Even in its intimate association with the cellulose micelles, it will not affect their anisotropy.

Ruthenium red and methylene blue were used as pectin stains, the former known to be the more specific. Reactions revealed a high concentration of pectin in the immediate cutinized zone outside the secondary walls, as well as in the middle lamellae. In removing these compounds the sections were treated in hot 5% KOH and again washed. Staining was repeated for checks with negative reactions in these pectin regions. Delignified sections were tested with the same results. Pectic compounds are known to be colloidal and isotropic. They appeared dark between crossed Nicols where there was no cellulose present.

To obtain an accurate determination of complete cutinization sections were left in Sudan III for an hour, which brought out the boundaries of the cutin more distinctly. Sections treated with chlorozinc iodide were examined to note the effect on the cutin layer and clues as to heterogeneity of structure were evident. Slow saponification was accomplished by heating sections on slides under cover glasses and slowly adding 10% KOH. The slide was examined progressively under the microscope as the saponification increased. A thin outer strip of the cutin layer was the only part of this layer to disappear, the inner portion which was already found to contain cellulose lamellae and pectin remaining intact. Since cutin is insoluble in cuprammonia, 72% sulfuric acid, and hot dilute acids and alkalies, those sections having gone through such reagents for other tests were rechecked and found to have the cutin still in place. The outer stratum of "pure" cutin was optically isotropic in all paraffin sections, but showed up anisotropically in some fresh sections. Weak double refraction in the inner stratum coincided with the pectin zone containing the cellulose lamellae. Although cutin can be optically isotropic or anistropic when free from cellulose, its anisotropy can be considered, and was here, as indicative of cellulose deposition.

It became evident, then, that these particular epidermal cell walls are of a complex nature in the orientation of wall constituents. Different degrees of lignification in xerophytic leaf structure are not uncommon but, to the knowledge of the writer, pits extending into the cutin layer are most unusual.