11. Criteria of Successful Processing

We have carried to completion the processing of some plant materials and have the finished slides before us. Our notebook contains a record of the entire process, from collecting the living plants to the completion of staining. We can now examine the slides critically and consider the criteria by which we may judge the success or failure of the processing. The severity of our scrutiny depends on the objectives of the study for which the preparations were made. We may be primarily interested in the distribution of tissue systems or the position of organs, paying little attention to the protoplasm. Rapid and convenient methods that preserve the desired structures with adequate accuracy need not be regarded as slovenly. Another study may require the preservation of the constituents of the protoplasm in their normal structure and position. The use of more elaborate, time-consuming processes is then justified.

It is axiomatic that slides for elementary students must be much more perfect than for advanced workers. Beginners waste much time puzzling over imperfections. They will draw faithfully a break produced in microtoming, the gap between the cell wall and the collapsed plasma membrane, or a speck of debris in the tissues.

The pathologist should be especially critical. Control slides of normal tissues must show cellular details with almost diagrammatic perfection in order to furnish a basis for interpreting pathological conditions. Studies in physiology, cytology, and experimental morphology also demand careful control of processing and critical examination of slides on the basis of some useful criteria.

The following illustrations were selected from well-known and widely used subjects. Leaves of different species exhibit different reactions to killing fluids. The quality of the fixation can be determined easily by the condition of the palisade cells. The outlines of the cells should not be wrinkled, and the chloroplasts should line the walls. Figure 11.1 *a* shows the excellent preservation of cellular and tissue details in soybean leaf, a highly spongy and therefore difficult subject, processed as outlined in the legend.

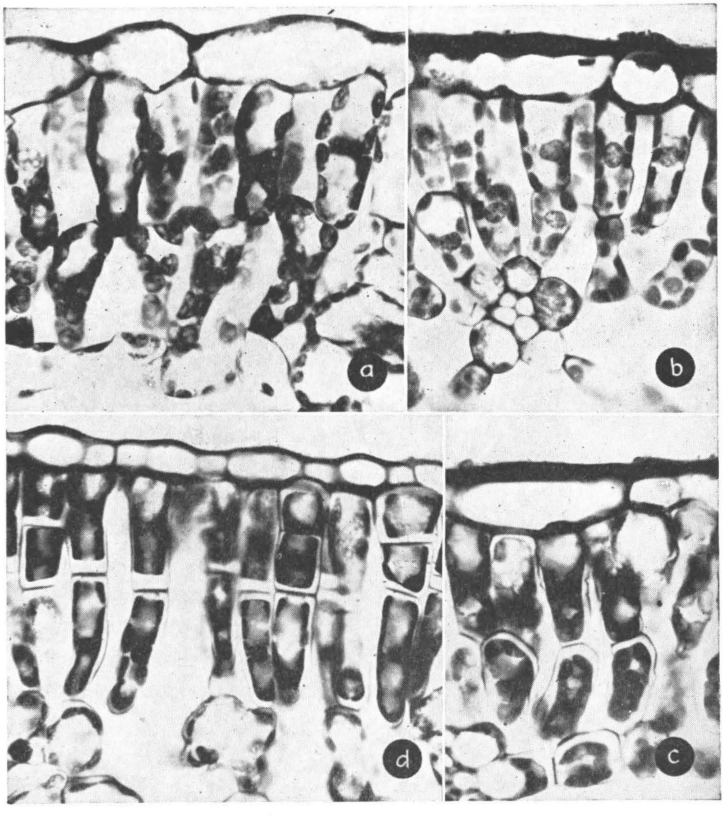

FIG. 11.1—Comparison of fixation in leaves: *a,* soybean, Craf III, acetone-tertiary butyl alcohol; *b,* cherry, turgid condition, Craf II, alcohol–xylene; *c,* cherry, plasmolyzed, *FAA,* alcohol–xylene; *d,* red ash, plasmolyzed, *FAA,* alcohol–xylene.

A striking illustration of the effects of different killing fluids is afforded by experiments with young leaves of cherry. Figure 11.1 *c* shows the severe plasmolysis obtained by killing in *FAA*. Figure 11.1 *b* shows the turgid, normal condition of the palisade and spongy parenchyma, following killing in a Nawaschin type formula, Craf II. After killing, both lots were processed simultaneously by identical methods, an alcohol-xylene series and careful infiltration in paraffin.

Stems are subject to the same defects as the leaves described above. Study Fig. 11.2 *a,* a cross section of alfalfa stem. Note that the delicate hypodermal chlorenchyma is intact, the cells have smooth, rounded outlines, and there is no marked cleavage between cells. Within each chlorenchyma cell the plasma membrane and the chloro-

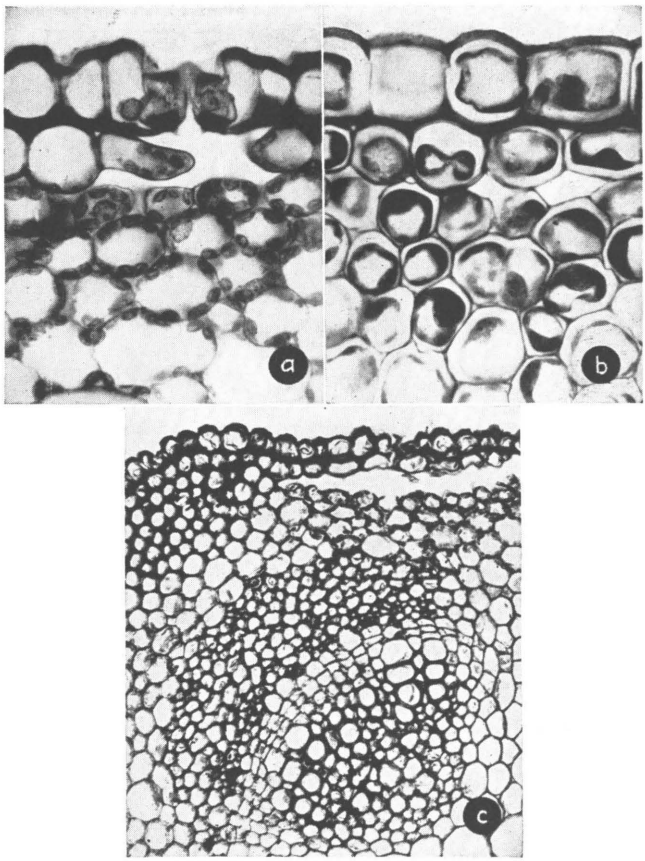

FIG. 11.2—Comparison of fixation in alfalfa stems: *a,* good fixation with Craf 0.20–1.0–10.0, dioxan process; *b,* plasmolyzed cells in cortical region, Craf 0.30–1.0–10.0, acetone-*n*-butyl alcohol process; *c,* good fixation, but with peeling epidermis, process as in *a.*

plasts are appressed against the cell wall, indicating absence of plasmolysis. The physical appearance of these cells may be regarded as approximating the structure of living cells. Observe further that the epidermis is not peeled away from the inner tissues. These features are indicative of successful processing.

In comparison with the above case, examine Fig. 11.2 *b,* also a cross section of alfalfa stem, killed in *FAA* and carried through an alcohol-xylene series. There is marked plasmolysis; the massed chloroplasts and cytoplasm absorb the stain and cannot be destained differ-

entially, producing harsh staining effects. Note especially the collapsed plasma membranes in the epidermis and chlorenchyma. This embedded material was brittle and only the pieces from the upper three internodes could be cut without serious tearing.

Figure 11.2 *c* is from a third batch of alfalfa stem which was processed exactly like the material from which Fig. 11.2 *a* was obtained. The structure of the deeper cortical cells is well preserved, but the epidermis shows extensive peeling away from the chlorenchyma. The condition of the protoplasts indicates that the killing fluid and subsequent processing were not at fault. It is probable that the peeling of the epidermis was caused by compression of the extremely soft hypodermal tissues when the fresh stem was cut into pieces for killing.

Roots are somewhat more difficult to judge than organs containing chloroplasts. Root cells that contain leucoplasts should be examined for the position of these plastids. If the plastids are inconspicuous, the condition of the thin plasma membrane will indicate whether plasmolysis has occurred.

The critic must be much more lenient in the examination of sections of wood. Boiling the wood in water, desilicification with hydrofluoric acid, and infiltration under pressure for long periods are not conducive to preservation of protoplasmic or even cell-wall details. Mechanical tearing can be easily recognized. In the nonliving elements (tracheae and tracheids) the concentric layers of the thick wall should not be separated. The innermost layer is often found to be completely separated in poor preparations. Parenchymatous elements, such as wood rays, diffuse wood parenchyma, and epithelial cells of resin canals, are subject to plasmolysis. Perfect fixation of these parenchymatous cells should not be expected.

The embryo sac of lily is a difficult subject that tests the effectiveness of a method and the skill of the worker. The young megasporocyte of lily is comparatively easy to preserve in good condition. Such fluids as chrome-acetic and Bouin's, followed by the traditional alcohol-xylene series, yield excellent preparations and a good ratio of well-preserved sporocytes. The sporocyte and integument initials in Fig. 11.3 are preserved very well indeed for routine class material.

With continued enlargement of the sporocyte the cytoplasm probably becomes highly fluid, the integuments elongate as thin sheets of tissue, and these structures become increasingly subject to damage. Look for evidence of plasmolysis of the sporocyte and wrinkling of the rims of the integuments. In Fig. 11.4 *a* the finely granular cytoplasm is obviously not shrunken, and the rounded rims of the integu-

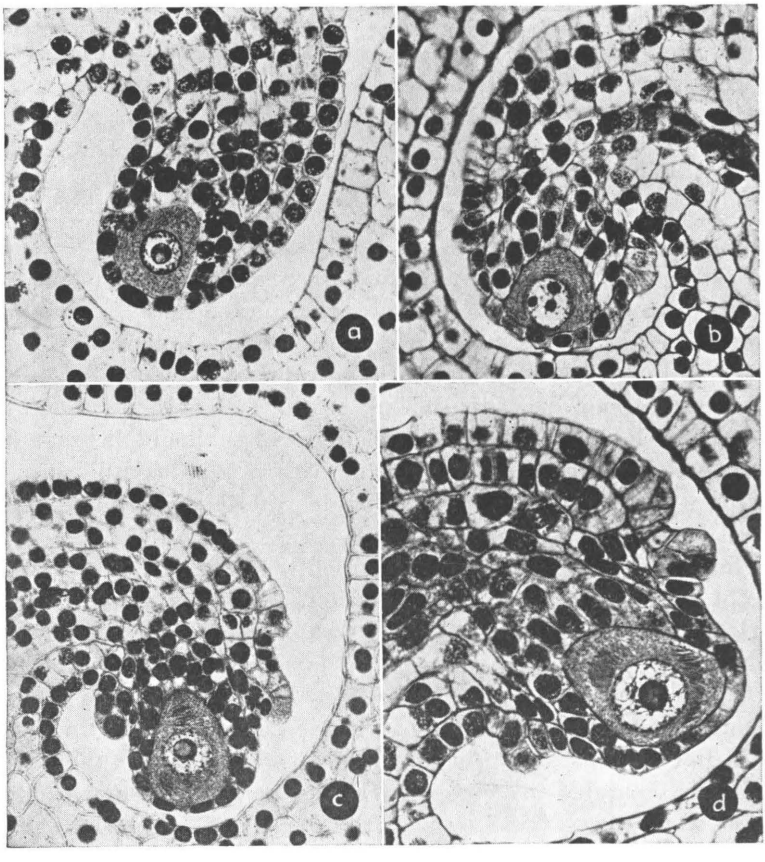

FIG. 11.3—Comparison of fixation in young ovules of *Lilium*: *a, L. speciosum,* chrome-acetic (0.5–0.5) 48 hr., alcohol-xylene; *b, L. tigrinum,* Allen-Bouin III, 4 days, three-grade dioxan; *c, L. speciosum,* chrome-acetic (0.5–0.5) 48 hr., alcohol-xylene; *d, L. tigrinum,* Allen-Bouin III, 6 months, acetone-tertiary butyl alcohol.

ments show very little distortion. Many readers will recognize an old acquaintance in Fig. 11.4 *b,* a typical Bouin, alcohol-xylene image that is frequently encountered in lily ovule. Although some batches prepared by this method have some good sporocytes, severe plasmolysis occurs frequently. However, nuclear fixation is usually excellent.

Figure 11.4 *c* shows very nearly perfect preservation of the sporocyte. The cytoplasm in Fig. 11.4 *d* is somewhat coarsely granular, but the chromosomes at the beginning of disjunction in the first division of meiosis are well fixed. Note that each chromosome is long and U-shaped, instead of a compact lump as with inferior fixation.

It is probable that undue blame has been placed on the killing fluid for the distortion of protoplasts of these large sporocytes. Recent developments in dehydrating and infiltrating methods indicate that these processes contribute much to the quality of the image. Rapid dehydration is probably one cause of distortion. The slow dehydration obtained by the glycerin evaporation method yields a very high ratio of well-preserved ovules. The mild dehydrating action of such paraffin solvents as the butyl alcohols and dioxan minimizes distortion of large sporocytes. It is probable that a properly balanced killing fluid,

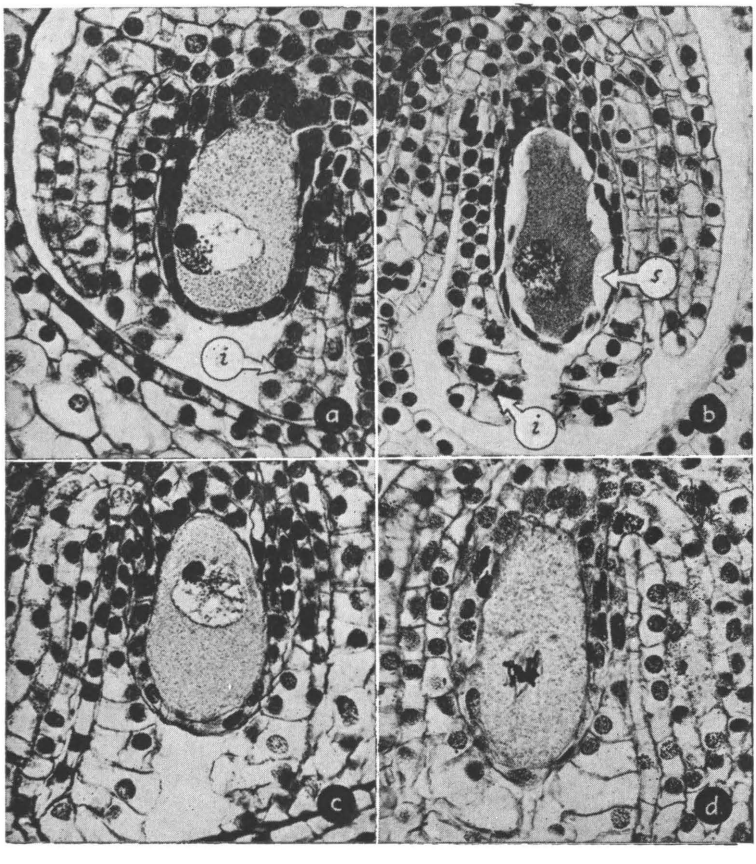

FIG. 11.4—Comparison of fixation in large megasporocytes of *Lilium*: *a, L. umbellatum,* Allen-Bouin III, 12 days, glycerin dehydration to alcohol-xylene; *b, L. speciosum,* Bouin, alcohol-xylene; *c, L. tigrinum,* Allen-Bouin II, 6 months, acetone-xylene; *d, L. tigrinum,* first division of meiosis, Allen-Bouin II, 6 months, acetone-xylene.

followed by a mild and slow dehydrating method, and progressive infiltration, yields the most satisfactory ratio of good ovules.

The foregoing discussion of the criteria of the quality of processed materials was illustrated by rather extreme cases of unsuccessful processing, contrasted with some decidedly choice materials. In actual practice it is not necessary to be so critical. A preparation having some plasmolysis may nevertheless be presentable and usable for the study of organogeny and tissue systems. At the risk of tiresome repetition it will be stated again that the choice of the subject, the technique used, and the quality of the finished product should suit the need.