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## Permanent Slides of Plant Cuticle Stained with Sudan IV and Sudan Black B

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## PERMANENT SLIDES OF PLANT CUTICLE STAINED WITH SUDAN IV AND SUDAN BLACK B

H. L. DEAN AND EDWARD SYBIL, JR.

Sudan IV is commonly used to stain fats, oils, suberin, and cutin. Materials stained in this dye are usually mounted temporarily in glycerine and are seldom kept as permanent slides. This may be due to the fact that balsam, clarite or similar mounting media, cannot be used to make permanent slides of preparations stained in Sudan IV. The dye is immediately removed by the xylene or toluene solvent of these media, leaving the preparations colorless. Thick fructose syrup and white Karo have been suggested as substitute mounting media and often make good mounts. A better mounting medium for this purpose is the commercial product, Clearcol. (Obtained from H. W. Clark, 5419 32nd St., N.W., Washington, D. C.) Oil soluble dyes such as Sudan IV, Sudan Black B, etc., are not removed from cutin or other substances stained by these dyes when this medium is used. Permanent mounts can be made of almost any material when Clearcol is used, and objects may be mounted directly from alcohol, glycerine or water. Better results are obtained when tissues are mounted from water. Paraffin sections are desirable when permanent slides are to be made of plant cuticle, but thin freehand sections may be used with success. Paraffin sections should be 7-10 microns thick for general utility slides. Sudan IV may be used alone to stain the cuticle, or a counter stain may be applied to differentiate cellulose walls from the cutin. The following abbreviated staining schedule for apple cuticle may be modified to apply to any type of cuticle.

### *SUDAN IV AND DELAFIELD'S HEMATOXYLIN*

1. Remove paraffin from sections and get slides into tap water. Wash well.
2. Stain in Delafield's hematoxylin, 5-20 minutes. Wash in tap water and examine under the microscope. If the stain is too dark or precipitates appear in the tissues, rinse 3-10 seconds in acidulated water. (1 or 2 drops concentrated HCL to 100 c.c. water). Wash *immediately* in tap water containing a few drops of a 0.1% aqueous solution of lithium carbonate. The

alkali restores the blue color of the hematoxylin, made reddish in the acid bath. For further details on the use of Delafield's hematoxylin see Dean (1940, 1941).

3. Wash well in tap water and pass through the alcohol series to 70% alcohol.
4. Stain 5-20 minutes in Sudan IV (.5% solution in 70% alcohol).
5. Rinse quickly in 50% alcohol and get into tap water. Wash well.
6. Mount in Clearcol.

The red color of the Sudan IV retained in the cutin contrasts well with the blue of the Delafield's hematoxylin in the cell walls, and a sharp differentiation is thus obtained between these parts. Slides made in this manner four years ago still retain their colors undimmed and the Clearcol remains water-clear over the sections.

Sudan Black B has recently been recommended (1932, 1935, 1939, 1940) as a precise stain for fat particles, especially in bacteria and leucocytes. Sharper staining is claimed for this dye than can be obtained with Sudan IV. Because this stain appeared to give such a good result with fat particles it was believed that it might also be used to stain suberin and cutin. Accordingly, a

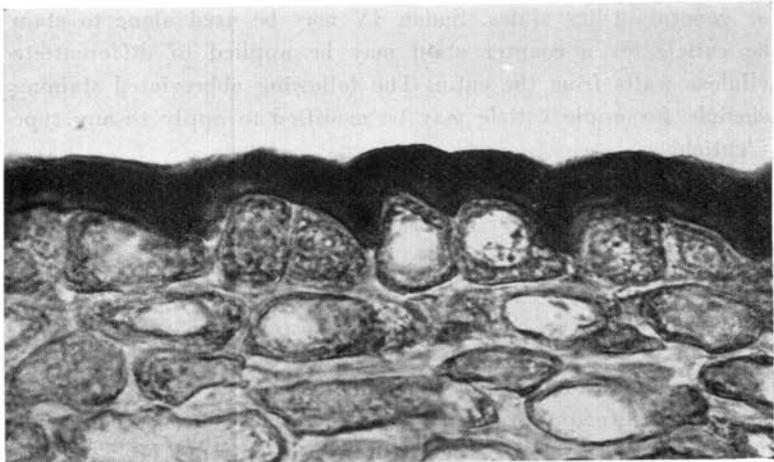


Fig. 1. Cuticle of gano apple stained in Sudan Black B, counterstained in Alizarin Red S.

series of experiments were made and the present note may be considered a preliminary report on the ability of this dye to stain cutin. Hartman (1940) recommended that this stain be dissolved in either 70% ethyl alcohol or in ethylene glycol. In the present study the following formula was developed and used for all work with this dye. Dissolve .2 gram of Sudan Black B in 100 cc. of 95% ethyl alcohol; to this solution add 50 cc. of glycerine, mix well and filter. This dye stains very rapidly and a stronger solution is not recommended for general use. Solutions of dye made in this manner are still effective seven months after mixing, and show no signs of deterioration. Previously (1935) this stain had been described as losing its efficiency in a short time. The following staining schedule is for paraffin sections of apple cuticle but may be modified for freehand sections. The dye may be used alone or with a counterstain.

#### Sudan Black B and Alizarin Red S

1. Remove paraffin and get sections into 70% alcohol. Wash well.
2. Stain in Sudan Black B, 10-25 seconds; rarely will it be necessary to stain as long as 40 seconds.
3. Rinse in 70% alcohol and get into tap water. Wash well.
4. Counterstain in 1% aqueous Alizarin Red S, 2-8 minutes. Alizarin Red S stains both cell contents and cell walls but this indiscriminate staining does not appear to affect the sharpness of the Sudan Black B.
5. Wash well in tap water to remove all surplus stain.
6. Mount in Clearcol.

A temporary counterstain may be applied instead of the Alizarin Red S. Stain in a 5% aqueous solution of Phloxine, 20-40 seconds. Wash well in water and mount in glycerine. This stain fades and cannot be used for permanent slides.

Sudan Black B stains cutin very sharply and is easily as precise in this respect as Sudan IV. Stained cutin has good visibility, and the blue-black color is better to photograph than a red-stained preparation of Sudan IV. Preliminary trials indicate that Sudan Black B is also effective as a stain for suberin. Experiments are still in progress with this dye and additional results will be reported later.

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LITERATURE CITED

- Dean, H. L. 1940. Delafield's Hematoxylin and Safranin for Staining Plant Materials. *Stain. Tech.* 15:61-65.
- 1941. An Improved Schedule for Staining Plant Tissues in Delafield's Hematoxylin and Safranin. *Chronica Botanica* 6:294-295.
- Hartman, T. L. 1940. The use of Sudan Black B as a Bacterial Fat Stain. *Stain Tech.* 15:23-28.
- Lison, L. 1934. Sur de nouveau colorants histologique specifiques des lipids. *Compt. Rend. Soc. de Biol.* 115:202.
- Lison, L., and J. Dagnelie. 1935. Methodes nouvelles de coloration de la Myeline. *Bull. d'Histol. Appl.* 12:85-91.
- Sheehan, H. L. 1939. The Staining of Leucocyte Granules by Sudan Black B. *Jour. Path. and Bact.* 49:580-581.