ment is held to a minimum in the larger pipettes by the following modifications:

Pointed capillary pipettes are made from tubing, 1.5 ± 0.2 mm. in external diameter, which is broken into lengths of about 13 cm.[2] The middle of each length is heated in a narrow flame, such as is made by a fishtail burner or the pilot of a Bunsen burner, and, when melted, drawn to a very fine point by quickly pulling on the two ends of the tubing. When cool, the very finely drawn glass is broken close to the conical tip; thus, two conically pointed pipettes are formed with fine openings about the diameter of a hair. To insure that these openings are patent, they may be inspected with the aid of a hand lens. With practice, three to four such pairs can be drawn at one time. The outer surface of these pipettes is cleaned with paper tissue, the pipettes then being placed in test tubes containing absorbent cotton on which the conical points rest. These containers are capped with unsized paper and then sterilized by dry heat, or by autoclaving followed by drying in an incubator.

For testing, the sterile pointed end of a pipette is dipped into the precipitating serum until a column of serum between 1.0 and 1.5 cm. long has been slowly drawn in by capillary action, then wiped with paper tissue and dipped into a drop of antigen solution until an equal amount has been drawn into the pipette. Air bubbles must not separate serum and antigen solution. This conical end is again wiped and plunged into a lump of plasticine in order to seal the hair-sized opening. The lower end of the pipette is pressed against a strip of plasticine, which has been previously placed on a block of wood, so that the pipette is held in a vertical position without being plunged into the plasticine. If it is mounted in this manner, the fluid in the upper part of the pipette will not be forced out by air pressure which would be developed were the lower open end forced directly into plasticine. The upper end should, however, be inspected to see whether any solution has been forced out, and if this has occurred, it should be wiped off so that no film will be deposited on the pipette.

If this procedure has been followed, the column of fluid will remain at the upper part of the pipette, the surface of which should be perfectly clean.

The above-outlined technique insures the minimal mixing of the underlying serum and overlying antigen solution; hence, there is a narrow zone in which the precipitate forms. When longer columns of serum and antigen are drawn into the capillary pipette, this interzone moves over a proportionally wider range, more mixing of the two reagents occurs, and the zone of precipitate is wider; hence, the reaction may be less intense. The same tendency to mixing of the two reagents exists when the reaction is set up, as previously advocated, in 1.5-cm. pipettes with both ends wide open: rapid mixing of the two reagents occurs, and the reactions may be so indeterminate that they often require confirmation in small test tubes.

Within 5 to 10 minutes a positive reaction is shown by a cloudy white disc of very fine precipitate at the junction of serum and antigen. With weak sera or antigens, a longer time may be required. When testing with streptococcal grouping sera, if the pipettes are placed in the incubator at 37° C. for an hour, weak cross-reactions with sera of other groups sometimes occur; hence, readings made within 5 to 10 minutes probably indicate more specifically the group to which the streptococci under examination belong. Upon standing, the precipitate formed early may redissolve, or it may clump, fall to the bottom of the serum, and be detectable the following morning after refrigeration.

Errors due to weak reactions may result from excessive mixing of the antigen and serum. Another source of error at times is to allow the test to stand too long before reading, because the precipitate formed early may redissolve. False readings may result from grease marks or other materials on the outer surface of the tubes over the zone of reaction. Antigens and sera should always be clear before preparing the test, since hazy sera, or sediment drawn into the pipettes with the reagents, may lead to false-positive readings.

[2] This capillary tubing is carried in stock by Arthur H. Thomas, Philadelphia.

References

1. LANCEFIELD, R. C. *Proc. Soc. exp. Biol. Med.*, 1938, **38**, 473.
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Warm Safranine for Plant Tissues

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In the course of recent botanical investigations conducted at the University of Michigan Biological Station, an attempt was made to decrease the length of time necessary for staining plant slide material with safranine. The slides and safranine were placed in a paraffin oven which was thermostatically controlled at 53° C., and a series of tests were made subjecting different materials of different thicknesses to varying lengths of time in the heated safranine. The procedure up to and following the safranine bath conformed to the norm.

The results indicated that completely satisfactory staining could be obtained in 15 minutes as compared with the 24- to 48-hour period necessary when the stain is used at room temperature. The color imparted by the warm stain is quite brilliant and remains fast in the tissue even through prolonged immersion in absolute alcohol. Young *Lycopodium* stem, ordinarily difficult to stain, showed excellent results after 15 minutes in the heated stain. Other material tested included hypocotyl of collard, stem of *Prenanthes alba*, and stem of *Diervilla Lonicera*.

Other tests showed that successful staining could be accomplished when the slides were left in the safranine for periods up to 3½ hours. However, material left in for 12 hours was overstained, resulting in a deep purple color which was not readily distinguishable, under low power, from the fast green used as a counterstain.

As the heat induces a rapid and thorough penetration of the dye, the excess stain is not as rapidly removed as under room temperature, and it is desirable to leave the slides in absolute alcohol for a somewhat longer time than is usual. For the same reason, the normal 1- to 2-minute immersion in fast green was increased to 3–10 minutes.

A brief examination of the literature indicates no previous mention of the possibilities of using warm safranine for botanical preparations, although Lee (*1*) mentions its use for zoological preparations by Griesbach in 1887.

Reference

1. LEE, B. *Microtomist's vade-mecum.* (9th ed.) Philadelphia: Blakiston, 1928.