

tions in the smears, greatly facilitating their interpretation. Attempts to simplify the technic as well as to avoid the necessity for relying on imported stains such as Ponceau de Xylidene and Light Green have since been carried out. These have been greatly facilitated by the report of Lillie³ that domestic Biebrich Scarlet and Fast Green FCF may be substituted for Ponceau de Xylidene and Light Green respectively; and that a mixture of equal parts of 5 per cent. phosphomolybdic and phosphotungstic acids gives adequate mordanting in one minute. On this basis, it has been possible to simplify and shorten the technic previously described for the vaginal smear and use domestic stains exclusively.

The revised staining technic embracing these modifications is as follows:

(1) From fixing solution, carry through alcohols to water; stain with Harris Hematoxylin for 2 minutes, and wash in running water for 5 minutes.

(2) Instead of the Ponceau de Xylidene-Acid Fuchsin-Orange G solution, 1 per cent. Biebrich Scarlet, water soluble (Nat'l Aniline and Chem. Co.) and 0.4 per cent. Orange G in 1 per cent. acetic acid. Stain 1 minute and rinse in water.

(3) In place of the 3 per cent. phosphotungstic acid mordant, a mixture of equal parts of 5 per cent. phosphomolybdic and phosphotungstic acids. Mordant 1 minute and rinse.

(4) In place of 0.3 per cent. Light Green, a 0.25 per cent. solution of Fast Green FCF (Nat'l Aniline and Chem. Co.) in 0.3 per cent. acetic acid. Stain 2 minutes. Do not rinse.

(5) Differentiate in 1 per cent. acetic acid for 1 minute, carry through alcohols to xylol and mount in damar.

It is possible to omit the hematoxylin stain under certain conditions, as in the routine treatment of the menopause with estrogens. With this omission, the smear can be stained in 5 minutes.

The assistance of Eugene J. Cohen in working out these modifications is gratefully acknowledged.

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SOLUTIONS OF CHLOROPHYLL IN SALT WATER

ALTHOUGH a number of workers have studied aqueous extracts of chlorophyll from fresh leaves, only Inman¹ seems to have discovered that the addition of salt to the water is beneficial. Since Inman seems never to have published his findings in this respect, and since the author hasn't time to do adequate re-

search with the method, it seems worth publishing this statement.

As various workers have stated, chlorophyll can be suspended in water if fresh leaves are ground in water, either with or without an abrasive. However, the suspended chlorophyll settles out within a few hours (with a few exceptions). Smith² has found that the addition to the colloid solution of a detergent will keep the chlorophyll in suspension. Less drastic treatment than that will stabilize the colloid. It is only necessary to grind the leaves with a salt and water solution rather than pure water.

Na₂SO₄ and NaCl have been found effective. The optimum concentration for NaCl is between 2 per cent. and 5 per cent. Since it has seemed desirable to control the pH, M/15 phosphate buffer of pH 7 is being used at present, and it gives very satisfactory solutions. CaCl₂ will not maintain the colloid in suspension. Buffers of pH 6 and below are not satisfactory, for the chlorophyll tends to decompose. Borate buffers at pH's 8 and 11 seem satisfactory, but it is feared that the high pH may change the chlorophyll in some way.

The chlorophyll suspension obtained in salt solutions is never clear. It possesses the various properties reported heretofore. It is relatively photostable, is precipitated by protein coagulants, passes through filter paper, is difficult to centrifuge down, has the red absorption band in the same place as that of an intact leaf, behaves as if negatively charged in cataphoresis, can be precipitated by ammonium sulfate and redissolved by addition of fresh buffer solution.

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² E. L. Smith, *SCIENCE*, 91: 199-200, 1940.

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³ *Stain Technology*, 15: 17, 1940.

¹ O. L. Inman and M. L. Crowell, *Plant Physiol.*, 14: 388-390, 1939; also in private conversation.