A New Cytological and Histological Fixing Fluid

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In the preparation of chromosomes for microscopic examination by the smear or squash technique, the Carnoy fixation[1] or one of its modifications is perhaps the most widely used by cytologists. It has, however, a number of disadvantages. The mixture should be freshly or at least recently made up before use; tissues cannot be stored in it for more than a few weeks; and, if it contains chloroform, the Feulgen reaction is inhibited with time. The frequent practice of storing materials in 70% ethanol after Carnoy fixation is, in our hands, only a desperate resort made necessary by the exigencies of the season or available research time and its use results in a rapid and steady deterioration of the stainability of the cells. As a result, the cytologist is the victim of a hectic cycle of feast and famine for research and teaching materials, and his choice of materials and prosecution of research are often seriously circumscribed.

This problem recently became acute with us during a study of avian chromosomes. Since only freshly killed material gave adequate staining reactions, the sacrifice of expensive birds at a frequency demanded by technical considerations might well have made the project too expensive for completion. An earlier effort to solve this problem by changing the staining combination (*1*), although an improvement, still left us with a chloroform-containing fixative which inhibited the Feulgen reaction and hardened the tissues after prolonged storage.

For these reasons a series of experiments combining a number of nonpolar or weakly polar organic compounds with propionic acid was run, using avian testes and 8-day-chick embryos as test materials for fixation. By an empirical process of examination, recombination, and elimination, the following formula (by volume) emerged: 6 parts isopropyl alcohol, 3 parts propionic acid, 1 part ether (petroleum), 1 part acetone, and 1 part dioxane.

This mixture is stable and can be made up in stock solution for use when needed, and it produces superb results. We have been using it in our laboratory for the past year in teaching and research and have found it eminently satisfactory for both plant and animal tissues. It has been used for avian testes, 40-hr and 8-day whole-chick embryos, the gonads of 4 mammals, grasshopper testes, and the following plant materials: *Tradescantia* and cucurbit microsporocytes, and root-tips of *Allium, Lilium,* and *Narcissis.* All the above materials, after storage in the fixative in the refrigerator at 4° C for periods of 5–11 months, are still staining sharply with propionic carmine and the Feulgen stain.

The time of hydrolysis for Feulgen in normal HCl at 60° C is 10 min for plant materials and 20 min for the animal tissues after prolonged storage.

With animal tissues the fixative is replaced once after 12–24 hr with fresh fluid, after which all materials are stored in the refrigerator until needed. These tissues can be either smeared directly or prepared for embedding in paraffin and the sectioning technique by replacing the fixative with absolute alcohol which is followed by xylol and paraffin. Thus all stages of dehydration save the one are eliminated, and we believe the fixation image is better than those usually produced by the conventional fluids containing salts of heavy metals. Furthermore, no bleaching is required prior to staining.

In preparation for hydrolysis for the Feulgen reaction, the tissues are washed for 1–2 hr, hydrolyzed, rinsed in distilled water, stained 2–3 hr in Feulgen, and smeared directly from Feulgen on the slide in a drop of 45% acetic acid. To make the Feulgen smear permanent, the slide is heated, pressure is applied on the coverslip, and the slide is immediately inverted in a Petri dish and covered with absolute alcohol to which fast green may be added for counterstain. When the coverslip comes off, it is immediately reunited to the slide with diaphane or euparal. The results, in our hands, are equivalent to any of the more complicated Feulgen procedures usually recommended in the literature. The same procedure may be used for making permanent preparations of propionic carmine smears.

[1] The original Carnoy fluid apparently consisted (by volume) of 6 parts absolute ethanol, 3 parts chloroform, and 1 part glacial acetic acid. Most investigators transpose the proportions between chloroform and acetic acid or omit the chloroform entirely.

Reference

1. NEWCOMER, E. H. *Stain Technol.,* **27**, 205 (1952).

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Comparative Effects of Total Body Radiation, Nitrogen Mustard, and Triethylene Melamine on the Hematopoietic System of Terminal Cancer Patients[1]

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Nitrogen mustard and allied agents have come to be considered as alternative agents for radiotherapy in the treatment of several forms of cancer. Comparable clinical regression of disease and similar histologic changes have led to the use of the term "radio-

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