A NEW METHOD FOR STAINING CELLS WITH COBALT AND BAL

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Cobaltous ions when used as a mordant form a colorless complex salt with the tissue constituents. The loci of cobalt complexes are rendered visible by developing them to a dark brown with BAL (2-3 mercaptopropanol).* Since a large amount of sulfur is present in the protoplasm and since cobalt has a high affinity for sulfur, we believe that the original complex formed when the tissue is mordanted in cobalt is a cobalt-sulfur complex. Another reason for believing that a cobalt-sulfur complex is formed is that a tissue treated as though prepared for an —SH test renders a better color.[1]

Compounds containing one —SH group do not form any color when cobalt is added unless the pH of the compound is 8.5 or higher. When a compound containing two or more —SH groups is added to cobalt a brown color is immediately obtained. H_2S and cobalt form a black sulfide.

This staining method is very useful for the fibrillar portions of the cytoplasm, especially for the staining of the mitotic spindle and centrioles. We wish to state that this is not a method for testing for the presence of —SH groups.

Fixation of material with trichloroacetic acid, 10 percent; formalin, 10 percent; Bouin's solution; Flemming-Strong solution; Allen's B-15 solution; Allen's B-20 solution; or Zenker's solution gives good results when stained by the cobalt-BAL method.

Blood for staining white cells is fixed in 100 percent alcohol 1 minute, dried quickly in air, and fixed in 10 percent trichloroacetic acid. The hot method of blood fixation by igniting the alcohol does not allow staining.

The procedure in general is as follows:

1. Fix and embed sections, mount material on slides (bleach if necessary with H_2O_2 as in Flemming-Strong technique).
2. Remove paraffin.
3. Hydrate to H_2O.
4. Place in 5 percent Zn $(Ac)_2$ 10 minutes.
5. Rinse 3 minutes.
6. Place in 3 to 5 percent Co $(NO_3)_2$ or any other cobaltous salt from 30 minutes to 12 hours.
7. Wash 1 to 30 minutes in running tap water.
8. Place in 35 percent alcohol containing 0.5 percent 2-3 mercaptopropanol. (*Note*: place the mercaptopropanol into 85 or 95 percent alcohol, then dilute to 35 percent alcohol and 0.5 percent BAL.)
9. Wash, dehydrate, clear, and mount.

If the material is placed alternately into cobalt and BAL, washing in water between cobalt and BAL

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* We are indebted to Hynson Wescott and Dunning, Inc., Baltimore, Maryland.

[1] Rapkine nitroprusside test for —SH groups. Techniques of Histo and Cytochemistry, Interscience Press, New York, 1949.

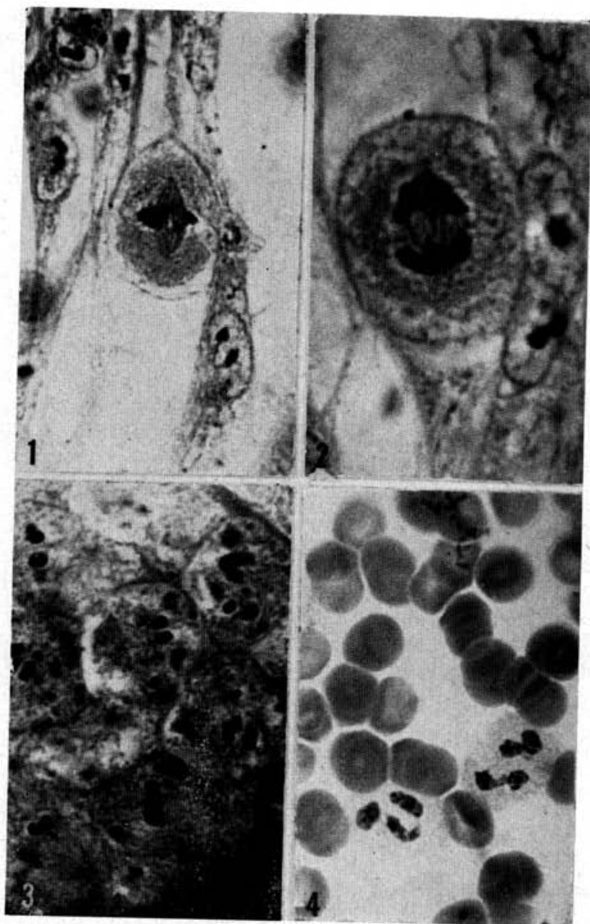

FIG. 1.—Metaphase spindle, tissue culture, chick heart fibroblasts.

FIG. 2.—Late anaphase, tissue culture, chick heart fibroblasts.

FIG. 3.—Late maturation division, grasshopper testis.

FIG. 4.—White cells, human blood.

and BAL and cobalt each time, the stain is strengthened infinitely.

Figures 1-4 are representative of this technique. The pictures were made using a micro-ipso attachment and 35-mm film.