A NEW NUCLEAR STAIN

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An extensively used nuclear stain is imported hematoxylin [2,3,5,6,7]. Attempts to replace it by Soviet nuclear stains have given reasonably good results, but the material suggested by the authors for obtaining the dye is not always available [1,3]. We have tried to prepare a cheaper nuclear stain from material widely available in the Soviet Union. Attention was directed to the pigment from seeds of the "fuksinka" variety of sunflower [4]. This pigment is soluble in water and is localized to the cork layer of the husk of the sunflower seeds [8, 9].

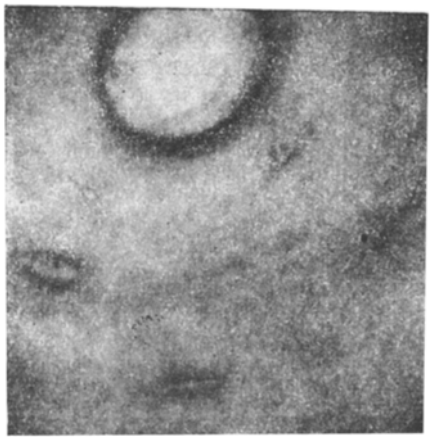

Fig. 1. Lamellar bone tissue. Decalcification with trichloroacetic acid. Fixation in 90° alcohol. Stained with dye from the husk of the "fuksinka" variety of sunflower, and counterstained with picro-indigocarmine. Magnification: eyepiece 7×; objective 90×.

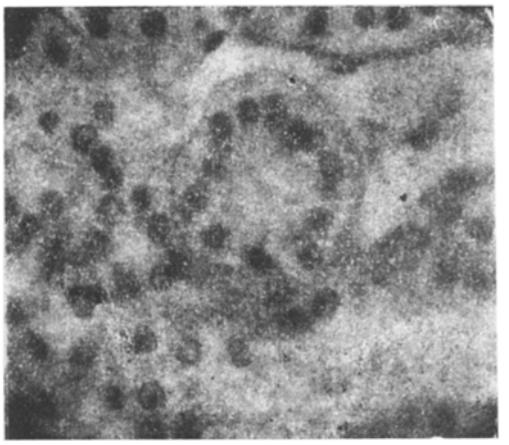

Fig. 2. Kidney. Fixation in 10% formalin (36 h). Stained with dye from the husk of the "fuksinka" variety of sunflower. Magnification: eyepiece 7×; objective 90×.

Production of the Dye. The husk of the sunflower seeds is separated from the kernel, chopped up finely, and placed in a glass vessel with distilled water. The mixture is allowed to stand for 20-30 min (the solution becomes cherry red) and the liquid is removed by evaporation at 37-40°. The resulting dry powder may be used for making solutions for staining. Fifteen grams of husk yields about 1 g of powdered dye.

Properties of the Dye. The well-dried extract is a blackish-violet powder, free from odor, and with a brackish, caustic taste. The powder is readily soluble in water, acids, alkalies, and slightly less soluble in 70°alcohol; it is insoluble in 96° alcohol, ether, benzene, and xylol. Depending on the pH of the medium, the color of the dye may vary. In a weakly alkaline medium, for instance, the dye solution is bluish-violet, but in a strongly alkaline solution it is brown. In an acid medium the dye solution is red in color. The powdered dye can withstand variations of temperature between +100° and −40° or lower, without losing its properties. An aqueous solution of the dye possesses weak staining properties. If the dye reacts with potassium alums, its staining properties are considerably enhanced. The prepared solution of the dye selectively stains cell nuclei, although it does also stain the cytoplasm very slightly.

Staining Method. An important factor when tissues are stained with this dye is the fixation. The tissue may be fixed in 96° alcohol, Zenker's formol, or Susa's mixture. However, the best results are obtained by fixing small pieces of tissue in 10-12% formalin for 24-48 h, not for longer because prolonged fixation in formalin lowers the quality of staining of the sections.

The staining solution is prepared by the following formula: distilled water, 50 ml; potassium alum, 2.5 g; dye, 0.95 g; glacial acetic acid, 12.5 ml; glycerol, 5 ml. The ingredients are dissolved in this order. The dye is ready immediately for staining sections. The stain solution may be kept in a closed glass bottle for several months without losing its staining properties.

Frozen, wax, and celloidin sections are stained with the dye solution (25-30 min), washed with distilled water, differentiated in 1% HCl in alcohol for a few seconds, transferred to a weak solution of ammonia (0.1-0.2 ml of 10% spirits of ammonia to 50 ml distilled water) until the sections turn blue, well washed in distilled water, dehydrated in alcohols of increasing strength, cleared in carbolxylol and xylol, and mounted in balsam. If necessary, the sections may be counterstained with acid dyes.

Sections may be stained with the dye solution (45 ml of distilled water, 5 ml 96° alcohol, 0.6 g dye) for 10 to 15 h after preliminary treatment (for 10 h) in a 2.5% solution of ferrous ammonium sulfate (as in Heidenhain's method [2,3,5,6]). Before staining, the sections are transferred from the mordant into distilled water for a short time. After staining, the sections are differentiated in a 2.5% solution of ferrous ammonium sulfate, washed in tap water for 10-15 min, dehydrated in increasing concentrations of alcohol, cleared in carboxylol and xylol, and mounted in balsam.

Results of Staining. A property of this dye is that it stains different tissues differently. In striped muscle fibers, stained a pale brown color, the nuclei appear black. The cross striations are readily seen. Connective tissue nuclei are stained black and the fibrous structures greyish-pink. The nuclei of epithelial cells are black, sometimes with a slight greenish hue. Their cytoplasm is pale pink. Erythrocytes in blood vessels are greenish-yellow in color, with a golden hue. Examples of the use of the stain are illustrated in Figs. 1 and 2.

SUMMARY

A new stain is suggested. A method of its preparation and staining technique are described. The stain is obtained from the husk of sunflower seeds of the "fuksinka" sort in the form of dry powder. The process involves aqueous extraction of the husk with subsequent water evaporation at 37-40°C. The stain obtained is mainly nuclear; however, it partly stains cytoplasmic structures in different colors.

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