Dehydration must be rapid so that cyton areas do not become completely destained. After washing in 80 per cent. alcohol the sections will become reddish in color with blue cyton areas. If the dehydration is properly carried out, the following structures will be selectively and permanently stained:

Cytons and Nissl granules deep blue; nuclei of blood vessel structures and neuroglia light blue; elastic fibers of blood vessels deep blue; erythrocytes pink; and neuroglia fibers light red.

The intensity of the cyton stain may be increased or decreased by varying the proportion of the Giemsa solution in the mixture.

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A RAPID METHOD FOR REMOVING COVER GLASSES OF MICROSCOPE SLIDES

IT is often necessary in cytological work to remove the cover glass of a slide, in order to replace a broken cover glass or restain the sections underneath. For this purpose most workers use xylene. The writer, however, has found that a mixture of 90 parts of xylene and 10 parts of n-butyl alcohol acts much more rapidly. The hard and brittle balsam or damar of old slides, which would require an immersion of several days in pure xylene, is usually dissolved by this mixture in a few hours. This time difference is probably due to the presence—especially in old slides—of a small amount of moisture in the mounting medium around the margin of the cover glass. Such moisture would offer a barrier to the penetration of pure xylene, but not to xylene containing n-butyl alcohol; for the latter is miscible with small amounts of water, as well as with xylene, balsam and damar. It should be remembered that butyl alcohol is a solvent of the aniline dyes, and so material stained with these substances will be destained in this xylene-butyl alcohol mixture.

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SPECIAL ARTICLES

X-RAY DIFFRACTIONS FROM HEMOGLOBIN AND OTHER CRYSTALLINE PROTEINS

SEVERAL attempts have been made during the past years to get x-ray diffraction photographs of the crystalline proteins. Most of these[1] have not been successful, but in a few instances very simple patterns have been observed.[2] These patterns, which always consisted of two rather broad and diffuse rings, have been found from proteins as different as edestin, excelsin, egg albumin and hemoglobin.

The diffuseness of the rings, combined with their simplicity irrespective of diffracting substance, suggests the pattern of a glass or other amorphous material rather than of a crystal. The probability that they are such amorphous patterns is strengthened by the recent statement[3] that a typical sharp line pattern can be prepared from a single crystal of pepsin left in its mother liquor.

We have been seeking to obtain truly crystalline powder patterns from edestin, excelsin and hemoglobin. Photographs prepared in the usual way from (1) commercial edestin, (2) well-crystallized edestin and excelsin freshly made from hemp seeds and Brazil nuts and (3) crystalline (white rat) oxy- and carbon monoxy-hemoglobin gave the familiar "amorphous" bands. When these preparations were examined microscopically they proved to be more or less completely altered after photography. Further microscopic study demonstrated that the protein crystals always decomposed rapidly on exposure to air. From the way this disintegration took place it was clear that they all contained water of crystallization which was very readily lost.

Photographs with copper K radiation were accordingly made of the wet crystals sealed into thin containers having windows of 0.01 mm glass. Under such conditions the protein crystals remain unchanged and typically crystalline patterns, consisting of fine, though very faint, lines, are produced. With this experimental arrangement reflections corresponding to large spacings lie too close to the central image for accurate measurement. Additional, and far more instructive, photographs have consequently been made with the longer chromium K radiation by keeping samples in moist chambers without protective windows. Some spacings thus measured on typical pictures of rat oxyhemoglobin are listed in Table I. There is no reason to believe that we have yet established the largest spacings that exist for this hemoglobin or for the other protein crystals. Our techniques, however, are being improved and it is expected that these maximum spacings will eventually be determined.

[1] For example, R. O. Herzog and W. Jancke, *Naturwiss.*, 9: 320, 1921; W. H. George, *Proc. Leeds Phil. Soc.*, 1: 412, 1929.

[2] See J. R. Katz, "Die Röntgenspektrographie als Untersuchungsmethode" (Berlin, 1931), p. 188.

[3] J. D. Bernal and D. Crowfoot, *Nature*, 133: 794, 1934.