

ingly, we think that wherever nature needs a mobile protein (serum albumin and globulin, secretions like milk-proteins, hormones like insulin, different enzymes, etc.) it applies the globular shape, and wherever it wants to build a solid structure it applies the rod shape. Proteins have been found to be mostly globular because unconsciously the mobile and more easily accessible proteins have been selected for study. The fibrous nature of proteins, having a mechanical function, has been recognized not because these are the only fibrous proteins, but because the mechanical function conditions a coaxial arrangement of the molecules, which arrangement makes the rod shape easily detectable.

If an animal tissue, like kidney, is extracted with weak saline, the mobile, globular proteins go into solution making about one third of the total protein. The remainder is insoluble in weak saline and represents the morphological structure, the solid edifice of the cell. This part of the protein is fibrous, as can be demonstrated by the intense double refraction of flow. In most tissues about half of this structure-protein can be brought into solution by extraction with Edsall's fluid (0,6 m KCl, 0,01 m  $\text{Na}_2\text{CO}_3$ , 0,04  $\text{NaHCO}_3$ ) containing 30 per cent. urea. The viscous, thixotropic protein can be precipitated by diluting (1:5) and neutralizing the solution. The protein, extracted in

this way, seems to form a definite fraction analogous to myosin. The protein also contains P in quantities comparable to the P of vegetable viruses. The rod shape and P content of these proteins invalidates two of the arguments brought forward for the oxogenous nature of certain viruses. Chloroplasts also contain fibrous proteins. The remainder of the protein, representing about one third of the original, can be brought into solution by treatment with 2 per cent. NaOH containing 30 per cent. urea at 60°. This fraction also shows a marked double refraction of flow.

By squirting the urea-salt solution into water the proteins can be pulled into threads, which, after drying, can be studied röntgenoscopically. The first measurements indicate that they are built on the same pattern as myosin or keratin.

Globular proteins, if treated with urea-salt or urea-NaOH give no double refraction of flow (serum albumin and globulin, casein, ovalbumin, lactalbumin, gelatin, edestin).

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### DOUBLE-STAINING *IN TOTO* WITH HEMATOXYLIN AND EOSIN

A METHOD of double-staining *in toto* with hematoxylin and eosin has been devised primarily for vertebrate embryos, but has also been applied to various vertebrate and invertebrate tissues with equal success. It is simple, reliable and a great time-saver when large numbers of sections, especially serial sections, are required. Furthermore, it eliminates the danger of losing any of the sections, which might easily occur if these were stained on the slide.

By using a modification of Ehrlich's hematoxylin as a *toto*-stain I have obtained excellent results with both embryological and histological materials of sizes up to 1 cm in diameter. The formula is as follows:

Ehrlich's hematoxylin, stock solution .....	8 cc
50 per cent. alcohol .....	30 cc
Glacial acetic acid .....	2 cc

This staining solution is highly penetrating, does not overstain and gives a practically pure nuclear stain if it is followed by a proper washing with very weak acetic acid. The entire procedure involved in this double-staining method is outlined below:

(1) Fix embryos or pieces of tissue in Bouin's fluid

for 1 to 3 days, according to size. Wash and preserve in 70 per cent. alcohol.

- (2) The yellow color in the tissue is removed by soaking in several changes of 70 per cent. alcohol saturated with  $\text{NaHCO}_3$  or  $\text{KHCO}_3$  (allowing about one hour for each millimeter of tissue). Then wash out the bicarbonate in 50 per cent. alcohol for one or more hours.
- (3) Stain in 20 vols. of modified Ehrlich's hematoxylin for 2 to 5 days (about one day for 2 mm of tissue). Shake from time to time to insure uniform penetration of stain from all sides of tissue.
- (4) After rinsing in water, soak tissue in several changes of 30 per cent. alcohol containing 0.5 per cent. acetic acid to extract the excess stain (about one hour for each millimeter of tissue). The last change of acetic alcohol should remain practically colorless after the tissue has been in it for half an hour.
- (5) Slow running tap water overnight.
- (6) Dehydrate through alcohols of 30, 50, 70, 85 and 95 per cent., 6 to 24 hours in each grade.
- (7) 95 per cent. alcohol with 0.3 per cent. eosin (alcohol-soluble), 12 to 24 hours.
- (8) Absolute alcohol with 0.3 per cent. eosin, 12 to 24 hours.
- (9a) For small embryos and soft tissues, clear in chloroform by the sinking method as follows:

In a small dish or a wide shell vial, pour a layer of chloroform on the bottom and then a layer of absolute alcohol with eosin on top of it. Next, place the embryos or pieces of tissue in the alcohol and allow them to sink slowly into the chloroform; leave for 2 to 6 hours or until they have sunk to the bottom.

- (9b) For large embryos and for tissues which become tough or brittle easily, clear in synthetic oil of wintergreen by the sinking method. Then replace the oil with chloroform in similar manner.
- (10) Chloroform saturated with paraffin overnight.
- (11) Melted paraffin, 2 or 3 baths, 1 to 3 hours in each bath. Embed in the usual way.
- (12) Cut sections, attach them to slides by the albumen-water method, and dry for 24 hours or longer.
- (13) Put sections in xylol to remove paraffin; bring them to absolute alcohol to differentiate the eosin, then back to xylol; mount in balsam.

Sections made from materials thus prepared are stained sharply and uniformly from center to periphery, and show good color differentiation similar to those stained on the slide. Among the tissues tried were pieces of Nereis, clam gill, grasshopper testis, Amphioxus, salamander liver and stomach, mammalian skin, pancreas, cartilage and growing bone, a 10 mm pig embryo and portions of a young shark having a diameter of 14 mm. Equally good results were obtained for all of them. In the grasshopper testis, the metaphase chromosomes and the spindle structure were nicely brought out but, as with the other alum-hematoxylin, except iron hematoxylin, the centrosomes were only faintly stained.

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#### STERILIZATION OF SURGICAL INSTRUMENTS BY DI-ETHYLENE GLYCOL

STERILIZATION of surgical instruments by boiling water involves disadvantages: the boiling point of water is relatively low; mineral deposits or stains may occur, especially if the water is hard; and there is a tendency for boiling water to dull the edges of cutting tools. Di-ethylene glycol is relatively free from these objections. While it may fume on heating, this is negligible when small sterilizers are used. If an electric sterilizer is set at "low," the temperature of the liquid rises to about 150° C. As its boiling point of 250° C. is approached, it fumes more markedly, but adequate sterilization should be obtained at temperatures where fuming is not apparent. On standing it evaporates very slowly so that only small amounts at infrequent intervals need be added to the sterilizer to keep it full. Di-ethylene glycol leaves neither stains nor deposits on the instruments. It has no appreciable dulling effect on cutting edges. Since it is completely soluble in water, di-ethylene glycol residues may be

promptly removed by dipping the heat-treated instruments in sterile water before use. Finally di-ethylene glycol is relatively inexpensive and readily available from chemical supply houses. These observations are based on two years' exhaustive trial with the compound in comparison with many others recommended for sterilization of dental instruments.

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#### "STUCK" SYRINGES

A SIMPLE and effective method of separating a "stuck" syringe is to place it in a container of concentrated nitric acid completely covering the syringe. Leave it immersed for a variable length of time, a week or two usually being sufficient.

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#### BOOKS RECEIVED

- BELL, HOWARD M. *Matching Youth and Jobs*. Pp. xiii + 277. Illustrated. American Council on Education, Washington. \$2.00.
- BERKMAN, SOPHIA, JACQUE C. MORRELL and GUSTAV EGLOFF. *Catalysis, Inorganic and Organic*. Pp. xi + 1130. Illustrated. Reinhold. \$18.00.
- BOORD, C. E., W. R. BRODE and R. G. BOSSERT. *Laboratory Outlines and Notebook for Organic Chemistry*. Pp. ix + 241. 28 figures. Wiley. \$1.75.
- BURGESS, PERRY. *Who Walks Alone*. Pp. viii + 308. Illustrated. Holt. \$2.75.
- COLLINS, A. FREDERICK. *Science on Parade*. Pp. xxiii + 314. 90 figures. Appleton-Century. \$3.00.
- Florida Academy of Sciences, *Proceedings for 1939*. Vol. 4. Pp. iv + 310. Illustrated. The Academy, Gainesville.
- GAUL, HARRIET A. and RUBY EISEMAN. *John Alfred Brashear*. Pp. viii + 220. University of Pennsylvania Press. \$2.25.
- GUGGENHEIM, M. *Die biogenen Amine*. Pp. xvi + 564. S. Karger, Basel, Switzerland. Sfr. 48.
- HART, HORNELL. *Chart for Happiness*. Pp. xi + 198. Macmillan. \$2.00.
- HUNTINGTON, ELLSWORTH. *Principles of Human Geography*. Fifth edition. Pp. xxiv + 594. Illustrated. Wiley. \$3.50.
- KRYNINE, PAUL D. *Petrology and Genesis of the Third Bradford Sand*. Pp. vi + 134. 37 figures. Pennsylvania State College. \$0.50.
- LUYET, B. J. and P. M. GEHENIO. *Life and Death at Low Temperatures*. Pp. 341. 33 figures. Biodynamica, Normandy, Missouri.
- NAMIAS, JEROME. *Air Mass and Isentropic Analysis*. Fifth revised edition. Edited by ROBERT G. STONE. Pp. vi + 232. Illustrated. American Meteorological Society, Milton, Mass. \$1.25.
- REISER, OLIVER L. *The Promise of Scientific Humanism*. Pp. xviii + 364. Oskar Piest, New York. \$4.00.
- ROBINSON, RAPHAEL M. *Stencils for Solving;  $x^2 \equiv a \pmod{m}$* . Pp. 14 and stencils. University of California Press.
- SHOHAT, J. A. and others. *A Bibliography on Orthogonal Polynomials*. Pp. ix + 204. National Research Council, Washington.
- STEFANSSON, VILHJALMUR. *Ultima Thule; Further Mysteries of the Arctic*. Pp. 383. 15 plates. Macmillan. \$3.50.