Staining Methods

The following list includes the staining methods used on the slides in the loan collection. It gives a brief sketch of their selectivity, mode of action, and procedure. They are arranged in alphabetical order for ready reference. The abbreviations are those used in the catalogue of the loan collection. You are not responsible for this material.

- **Acid phosphatase reaction**: This histochemical technique is used to recognize lysosomes due to their acid phosphatase content. Sections are incubated in a solution containing a lead phosphate. The phosphate is released by enzymatic activity of acid phosphatase (lysosomal enzyme) and is precipitated as lead phosphate, and is then converted to lead sulfide a black deposit.

- **Alkaline phosphatase (Al. P)**: The histochemical technique used for demonstrating the enzyme, alkaline phosphatase, blackens the cells and tissue containing the enzyme. In general, the degree of blackness is correlated with the quantity of enzyme present. Exact localization is complicated by the fact that the enzyme may shift its intracellular position during the histological procedure. Sections are incubated in a solution consisting of sodium glycerophosphate and calcium nitrate. Through the action of the phosphatase, calcium phosphate is precipitated in those regions where the enzyme is present. For visualization in sections, the calcium phosphate is converted into cobalt phosphate and finally into cobalt sulfide, which is black.

- **Azocarmine**: Nuclei are deep red; cytoplasm is a pale red.

- **Azure II - Eosin (Az. II. E.)**: Nuclei are blue or purple. Basophilic material blue. Acidophilic material red. Red blood corpuscles orange.

- **Berlin Blue (Prussian Blue)**: An insoluble particulate iron-cyanide compound, which is used for the injection of blood and lymph vessels.

- **Best's Carmine**: A specific stain for glycogen by which the glycogen granules are stained red. The PAS (periodic acid Schiff reaction) also colors glycogen red and is more commonly used.

- **Bielschowsky's Silver Method**: The reticular connective tissue fibrils are black. All other structures, yellow or brown. An impregnation method, which depends on the reduction by formalin of the easily reducible silver salt, silver ammonium hydroxide.

- **Bodian Silver method**: Metallic silver is precipitated by the action of a reducing agent (either exogenous or endogenous). The exogenous agent results in deposits on reticular fibers and portions of the junctional complex (argyrophilia). An endogenous agent results in precipitation on granules of enteroendocrine cells (the argentaffin reaction).

- **Cajal's Silver Stain (Cajal)**: Neurofibrils, axons and dendrites black. Other parts brown. The general principle of the Cajal methods (and there are many modification) is the application of photographic developers to tissues, which have been treated with silver nitrate.

- **Chrome Hematoxylin and Phloxin**: The use of these dyes for the differential staining of the alpha and beta cells of the islets of Langerhans was described by Gomori, 1941 (Am. J. Path., Vol. 17). The granules of the beta cells are stained a deep blue; those of the alpha are pink or red. The D cells are not differentially colored.
• **Cresyl violet**: A basic dye. See Nissl.

• **Eosin (E)**: An acid dye. Colors cytoplasm red; red blood cells, orange. Used as a counter-stain. See under H&E.

• **Foots Silver**: This is a modification of Bielschowsky's silver method. The thin collagen or reticular fibers stain black, other tissues remain pale. Azocarmine is frequently used as a counter-stain to color the cells and collagenous tissue red.

• **Giemsas**: Methylene blue eosinate, azure B eosinate, azure A eosinate, methylene blue chloride in methanol. Golgi Silver Method

• **(Golgi)**: A black deposit of reduced silver is laid down on the surfaces of nerve cells and neuroglia cells so that the form of the cell body and its processes stand out prominently in an almost colorless background. Only single cells here and there are selected by the stain. The method consists essentially of immersing fresh pieces of nervous tissue first in a solution of potassium dichromate (and usually osmic acid also) and then in silver nitrate.

• **Hematoxylin and Eosin (H&E)**: Hematoxylin is not a true basic dye. It is used with an intermediary, which recognizes anionic tissue components. Hematoxylin is nearly a specific stain for chromatin and it is therefore referred to as a "Basic" stain. It stains the nuclear network, chromosomes, etc., blue. It is a regressive stain and is extracted by very dilute acid or acid alcohol. It may be used after almost any fixative and is a permanent stain.

Eosin is a red general cytoplasmic stain. It combines with hemoglobin to give an orange color. It is an acid dye and the terms acidophilic, oxyphilic and eosinophilic are often used interchangeably. It may be used after any fixative and is used as a counter-stain in many combinations in addition to hematoxylin.

• **Hematoxylin and Orange "G" (H & Or. G.)**: Orange G. is substituted for eosin. Acid orange-G specifically stains the granules of acidophilic cells of the adenoypophysis.

• **Hematoxylin, Picric Acid and Acid Fuchsin (H & P.A.F.)**: See van Gieson.

• **Heidenhains Iron Hematoxylin**: Chromatin material (nuclear network and chromosomes) blue black. It is a popular cytological stain, especially for the study of mitosis. It can be used after almost any fixative.

• **Injection (Inj.)**: The channels in the tissues, for example, blood and lymph vessels, are injected with a colored mass. Berlin blue in dilute gelatin is a commonly used mass.

• **Mallory Azan**: Collagenous fibers are blue. Nuclei are red. Cytoplasmic staining varies from pink to reddish brown, depending upon the cell type.

• **Masson's Trichrome Stain (Masson's Tri.)**: Collagenous fibers blue. Muscle red. Nuclei red. The sections are first stained with hematoxylin. They are then treated with ponceau red and acid fuchsin, phosphomolybdic acid and aniline blue. You should be aware that in other laboratories light green is used in place of aniline blue.

• **Methylene blue**: A basic dye. See Nissl.

• **Nissl**: A method of staining nucleic acids (e.g. ribosomes, RER, heterochromatin, nucleoli). A dye such as methylene blue, toluidine blue or cresyl violet is used. Orange "G" (Or. G.): A general cytoplasmic stain similar to eosin in action. Stains cytoplasm yellow or orange.

• **Osmic Acid or Osmium Tetroxide (OsO4)**: A selective stain for unsaturated lipids and for lipoproteins such as myelin, which it stains black. Also used as a fixative, especially for electron microscopy.

• **Periodic Acid Schiff and Hematoxylin (P.A.S. & H.)**: Carbohydrates and carbohydrate compounds may be demonstrated by this histochemical technique. Most carbohydrates react with periodic acid to produce aldehydes, which convert the colorless Schiff reagent to pink, or magenta. Hematoxylin or methyl green is used to stain the nuclei. Glycogen, mucin, elastic fibers, reticular fibers, basement membranes, thyroid colloid, basophilic granules in the pituitary gland, and other polysaccharides such as the ground substance of cartilage are stained fuchsia or pink.
- **Phloxin:** This is a cytoplasmic stain belonging to the eosin series of dyes. It gives a reddish tone to the cytoplasm.

- **Mallory's phosphotungstic Acid Hematoxylin (Phostung. Hem.):** Nuclei, muscle fibrils, collagenous fibers, matrix of bone and cartilage are pale red. Astrocytic glial fibrils are blue. Best after Zenker fixation.

- **Regaud's Hematoxylin for Mitochondria:** Among the many methods used to demonstrate mitochondria by light microscopy, the most permanent and the simplest is Regaud's modification of iron hematoxylin on sections of material fixed in potassium dichromate and formalin and subsequently mordanted in dichromate. After staining, the slides are differentiated to remove the hematoxylin from most cytoplasmic components other than mitochondria. Unfortunately, the results are not uniform: some cells will be over-stained and some under-stained. Therefore a number of microscopic fields should be examined.

- **Silver nitrate (Ag.):** The intercellular cement substance of epithelium is black. This is an impregnation method. The fresh tissue is treated with silver nitrate and exposed to strong light, which reduces the silver.

- **Silver Method for Golgi complex:** Many methods have been used for staining the Golgi complex of the cell. One of the best methods consists of direct fixation of fresh tissue in a solution of silver nitrate in formalin, development in hydroquinone-formalin, followed by the usual procedure for paraffin embedding and sectioning. In the slides prepared for the class sets, the nuclei of the cells have been stained lightly by azocarmine.

- **Sudan Black:** This is a stain that colors fat droplets black. There are several Sudan dyes, among which are Sudan III and Sudan IV (Scarlet R.). These stain fat droplets red as does Oil-red-O.

- **Supravital staining:** A vital stain (e.g., trypan blue) is applied to an animal in life; a supravital stain (e.g., Janus green, neutral red) is one that is applied to cells or tissues removed from the body. See the first laboratory exercise, Introduction: Microscopy - Cytology for a description of the properties of neutral red and Janus green vis a vis particular subcellular organelles.

- **Toluidine blue:** A basic dye. See Nissl.

- **Van Gieson:** Collagenous fibers are yellow (e.g., dense connective tissue and bone), cartilage matrix is brown. Elastic Tissue Stain and Van Gieson's Picric Acid Fuchsin (Vieh. Van G. Verhoeff's): In Verhoeff's stain the elastic tissue is black and in this combination with Van Gieson collagenous tissue is red.

- **Weigert's Hematoxylin (W. Hem.):** A modification of Heidenhain's iron hematoxylin. Stains the myelin sheath black.

- **Weigert's Elastic Tissue Stain:** Elastic fibers purple or black. It is a specially prepared combination of basic fuchsin, resorcin, ferric chloride, water and alcohol.

- **Wright's Blood Stain (Wright):** Nuclei blue or purple. Basophilic granules blue, acidophilic granules red, and neutrophilic granules reddish lilac. Red blood corpuscles orange. The eosinates of polychromed methylene blue are dissolved in absolute methyl alcohol. When this solution is placed on a dried blood smear, the methyl alcohol acts as the fixative, and the dissolved dye begins the staining process. After from one to three minutes, the stain is diluted with an equal volume of distilled water. This differentially stains the cytoplasmic granules and is allowed to act for about three minutes. It is then poured off and the preparation is washed briefly in tap water and allowed to dry.

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