Save That Dye!

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All laboratory workers face the ongoing requirement that all chemicals and solutions used in the lab be labeled with the date of receipt or preparation, together with the expiration date of the chemical and the initials of the preparer, as well as any known hazards associated with that chemical. Officials from accrediting agencies, sometimes unannounced, pick and poke around laboratories looking for outdated chemicals and solutions, including biological stains left unsuspectingly at the back of a fridge or on a seldom used shelf. If they find such an outdated stain or chemical a citation can result that must be addressed before further accreditation is given. This has resulted in a knee jerk reaction in many laboratories, with a good many dyes and stains discarded before their time. (For the purposes of this article, dyes refer to the dry powders and stains refer to staining solutions, ready to use.) Surprisingly, the dyes kept on hand in histology for the preparation of staining solutions, for the most part, have no expiration date and can be kept for long periods. However, that is not to say that all dyes keep for ever, or that unstable dyes can be kept for long periods. And most certainly a dye that did not work when first purchased will not improve with age!

To appreciate the longevity of histological dyes, some knowledge of the history of dye manufacture is needed. The dye industry was created in 1856 in London when William Perkin created the color mauve from aniline while attempting to synthesize quinine. Aniline was available in large quantities from coal tar, a by-product of the recently created coal gas industry. Following Perkin's discovery, dye industries sprung up around Western Europe, usually in industrial areas close to the site of the coal gas industry and close to a river. Companies we now associate with pharmaceuticals such as Ciba, Giegy, Hochst, Agfa and Bayer were prominent or even founded during the early period of the dye industry.

The major use of dyes then, as now, was in staining of fabrics. Large sums of money could be made by a manufacturer of a new color dye that was "fast" *i.e.*, that bound well to the fabric and resisted fading from the sun or repeated washings. Many of the techniques familiar to histologists were first used to bind dyes to fabrics. Pre and post Mordanting, auxiliary chemicals and heat application to enhance staining were first used in the dye industry[1].

Competition was fierce. When a new dye was patented, others in the field would prepare the dye and study its structure, then prepare the dye by a different chemical method thus avoiding patent infringement. Or perhaps a slightly different hue was obtained by slight changes in the molecular structure. Other differences were created during manufacture. The solid dyes were "salted" out of solution by different methods and other chemicals were added to the dyes to stabilize the staining of fabrics. Rather confusing to us now is the names used for dyes and the use of synonyms. Often the dye was named after a place or a flower of a plant whose color the dye resembled. However, if the dye was made by another company under patent, or by a slightly different method, the dye might be given another name. Lillie[2] reports that some dyes can have up to 40 synonyms. And then, some dyes were just named incorrectly!

Biologists of the time such as Ehrlich, Schmorl and Unna searched through the hundreds of newly available dyes looking for those that had applications in the staining of biological tissues.

Soon companies sprung up supplying these new dyes to biologists. The most famous of these was the Grübler Company based in Leipzig, Germany. George Grübler the founder obtained samples of dyes from the new industry, and using secret empirical methods tested them on biological tissues for their use in science. Those he found acceptable he purchased in bulk, repackaged, and sold to biologists. Soon Grübler dyes developed a reputation as the best dyes for use in biological studies. Prominent scientists of that time recommended Grübler dyes in their writings. Van Gieson for example recommended Grübler dyes, as did Mallory[3].

World Wars I and II created an embargo on German dyes and spurred the development of the dye industry in the United States. The first dyes manufactured in America were often inferior to the German dyes and concerned scientists created the Biological Stain Commission to test and certify biological stains for use. In more recent years the Society of Dyers and Colourists in Bradford, England have created the color index number system based on the dyes chemical structure. Between them, these two organizations have assisted histologists by standardizing the dyes used to stain biological tissues.

A few years ago this writer was given a box of old dyes, mainly Grübler with a few Aniline Dye Company samples that had been purchased at an auction of medical memorabilia. More recently other Grübler and National Aniline & Chemical Company samples have been donated. Some of these dyes had never been opened. Others were in partially filled bottles. The Grübler dyes were prepared between 1880 and 1914. A study was undertaken to compare these dyes with modern day equivalents. Fourteen were selected for the study. These dyes were studied using spectrophotometry and in a variety of histological special stains

in common use today. Where possible, modern day dyes were used as controls.

The diamond fuchsin and fuchsin-bacillus dyes were both samples of basic fuchsin. Both samples had a maximum absorbance of 548 nm. They both stained well in the Ziehl Neelsen method for acid fast bacteria. When both were used to prepare Schiff's reagent for the PAS reaction, the results were inferior to modern day samples of basic fuchsin.

A Sudan III sample had a maximum absorbance of 509 nm in alcohol and stained lipid material in frozen sections of adrenal gland, but a paler shade of red than the modern day oil red O used as a control. Scarlet R is one of the names that has had different uses in the industrial dye industry. This sample dissolved in organic solvents and was originally thought to be Sudan IV. It dissolved in organic solvents and gave only a pale, off red coloration to lipids in frozen sections. Its maximum absorbance in alcohol was 513 nm. An orange G sample had an identical spectral curve to the modern day control (479 nm) and gave excellent results in the Mallory trichrome stain and the PAS-hematoxylin-orange G method for acidophil cells in the anterior pituitary. An aniline blue had a spectral absorbance of 595 nm and gave excellent results in the Mallory trichrome method.

The Pyronin sample had a maximum absorbance of 549 nm and spectral curve that most closely resembled that of pyronin Y. When used in the methyl green-pyronin Y method for RNA and DNA, this sample gave the best results with Carnoy-fixed material. The fuchsin S acid and rubin S were both examples of acid fuchsin and had close maximum spectral absorbencies (fuchsin S 546 nm, rubin S 550 nm) and similar spectral curves with the fuchsin S sample having a shoulder at 500 nm. Lillie[2] discusses this dye

in detail and states that the dye usually contains varying amounts of rosaniline, pararosaniline, magenta II and new fuchsin. These samples probably contained different amounts of these constituents. A safranin O water soluble and safranin yellow water soluble samples both had a maximum absorbance at 532 nm and identical spectra. Both gave adequate results in the Gram stain (Lillie) and stained umbilical cord matachromatically. A Grübler carmine sample had the typical bright red carmine color and was the natural dye obtained from the insect, Coccus cacti. This dye gave adequate results with the best carmine method for glycogen and the lithium carmine method for nuclei. Aurantia is an obsolete textile dye related to picric acid. Lillie[2] describes it as poisonous and explosive. When used in place of the picric acid in the Van Gieson method, connective tissues stained bright red orange with yellow cytoplasm. Its absorbance was 415 nm in alcohol.

Hematein is a naturally occurring dye extracted from the logwood tree, Haematoxylon campecianum. It is the oxidized form of hematoxylin. This sample gave poor results with the Mallory phosphotungstic acid hematoxylin and Heidenhain iron hematoxylin methods compared to the modern day control. A methyl eosin was assumed to be a member of the eosin group of dyes of which the alcohol soluble eosin Y (C.I. 45380) is most popular today. This sample dissolved poorly in water and gave bland results when used in a routine hematoxylin and eosin method. Only strongly acidophilic tissues stained with the dye. The graduated shades of pink associated with good eosin staining were missing.

Conclusion

Biological dyes can retain their staining characteristics up to one hundred years after manufacture, if kept sealed and in a cool, dry atmosphere. There are some caveats to this statement. Stains that did not work well immediately after manufacture will certainly not work well after long storage. Also dyes or mixtures of dyes and other chemicals in which there are ongoing chemical reactions such as oxidation may well become inactivated after time. The Romanowsky type dyes such as Giemsa, Leishman and Wright also involve mixtures of dyes and chemicals where optimum reaction time may have passed by.

However, for all old biological dyes, histologists are urged to try them out using good control material and see their appearance in stained control tissue sections before making a decision to throw them out[4]. The development of the dye manufacturing industry is an interesting subject for the histologist interested in history[1]. ■

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