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The Use of Vital Dyes in Ocular Surgery

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Abstract. Vital dyes have advanced diagnosis and surgical technique in various specialties, including oncology, gastroenterology, and ophthalmology. In ocular surgery vital dyes are widely used in cataract and vitreoretinal surgery. Worldwide, intra-operative use of trypan blue during cataract surgery has enhanced visualization of the anterior capsule during capsulorrhexis, and patent blue has been recently licensed in Europe for cataract surgery. For chromovitrectomy, the vital dyes indocyanine green, infracyanine green, and brilliant blue stain the internal limiting membrane, and trypan blue and triamcinolone acetonide help visualize epiretinal membranes and vitreous, respectively. Intra-operative vital dyes are finding uses in corneal, glaucoma, orbit, strabismus, and conjunctival surgery. We provide a summary of current knowledge of the use of vital dyes in ocular surgery. We review the properties of dyes, techniques of application, indications, and complications in ocular surgery. Vital dyes represent an expanding area of research, and novel dyes deserve further investigation. (**Surv Ophthalmol** 54:576–617, 2009.

Key words. vital dye • staining • retina • trypan blue • indocyanine green • vitrectomy • chromovitrectomy

Introduction

Dyes are chemical compounds that bind to various substances in nature to induce color.[178] When dyes color living tissues or cells, they are called *vital dyes*.[331] Vital dyes emerged recently as important and effective surgical adjuvants to enhance visualization of ocular tissues. In cataract surgery the blue dye trypan blue (TB) gained widespread use because it stains the anterior capsule and enables easier intra-operative removal of this fine, semi-transparent membrane.[153] In vitreoretinal surgery, greening and bluish vital dyes such as indocyanine green (ICG) and brilliant blue (BriB) also facilitated visualization and removal of pre-retinal membranes as a result of their different affinities to intraocular collagen and cellular elements.[306,309] Vital dyes has also been used in corneal, glaucoma, orbit, strabismus, and conjunctival surgery. We discuss the use of vital dyes in ocular surgery, including their biochemistry and pharmacology, their clinical effectiveness in staining ocular tissues, and the surgical techniques that utilize vital dyes, as well as clinical safety and toxicity issues.

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Biochemistry, Pharmacology, and General Information of Vital Dyes Used in Ocular Surgery

Vital staining refers to the coloration of living cells or tissues. When vital staining is done in a living organism, it may be called *intravital staining*, whereas *supravital staining* is defined as the application of dyes to living cells or tissues freshly removed from the body.[178] The various dyes currently available may be classified according to their pH, solubility, source, or staining property. A commonly used classification of vital dyes according to their chemistry is applied in this section.

AZO DYES

Azo dyes are large class of synthetic organic dyes that contain nitrogen in the azo form (-N = N-) in their molecular structures connecting aromatic ring compounds. Azo dyes give bright, high intensity colors, and their biggest advantage is cost-effectiveness. Because these can be easily chemically altered, an enormous range is available, constituting 60% of all synthetic dyes.[219]

TB is an anionic hydrophilic azo dye with the formula $C_{34}H_{24}N_6Na_4O_{14}S_4$ and a molecular weight of 960 daltons. Live cells/tissues with intact cell membranes usually are not colored because their selective control of cellular membrane transport does not allow binding of TB.[49] TB has been widely used in both vitrectomy and cataract surgery. TB is commercially available in an 0.15% concentration for vitreoretinal surgery under the brand name Membrane Blue (DORC International, Zuidland, Netherlands) and as Vision Blue in a 0.06% concentration for cataract surgery (DORC International). TB as Membrane Blue and Vision Blue comes in a solution containing small amount of sodium salts, 8.2 mg of NaCl, and water. The osmolarity of Vision Blue ranges from 257 to 314 mOsm/Kg and the pH from 7.3 to 7.6.[400,404]

Janus green (JG) is a basic azo dye of chemical formula $C_{30}H_{31}N_6Cl$ used histologically to stain mitochondria supravitally. JG changes color according to the amount of oxygen present. For ocular surgery, JG has been used in the supravital staining of corneal endothelium to assess viability before keratoplasty; however, the dye is not utilized intraoperatively.[300]

ARYLMETHANE DYES

Arylmethane dyes containin one carbon linked to two benzene or naphthalene groups bound to one moiety of N or O and one amino group.[169] The variable substitution of rings in the amino group determines further subclassification of this group of dyes, with four recognized families: diarylmethanes, aminotriarylmethanes, hydroxytriarylmethanes, and hydroxyaminotriarylmethanes.[178]

Gentian violet (GV), also known as crystal violet or methyl violet, is a water-soluble cationic aminotriarylmethane dye used mainly in histological preparations. This purple dye has a molecular formula of $C_{25}H_{30}ClN_3$ and molecular weight of 407 daltons.[178,377] GV is also used for the treatment of burns and other injuries to the skin and gums in a weak (1%) aqueous solution. In ophthalmology GV has been applied for anterior capsule visualization and as a marker of the cornea and conjunctiva.

BriB, also named Acid Blue or Coomassie, is a blue anionic aminotriarylmethane chemical compound which has the chemical formula of $C_{47}H_{48}N_3S_2O_7Na$ and a molecular weight of 854 daltons.[397] The synthetic blue agent has been certified as a food additive in Europe and may be used as a protein marker in cardiovascular and neurologic diseases.[67] Animal and human data on the use of BriB during vitreoretinal surgery and for anterior capsule staining have been described in recent years, resulting in its approval for intraocular use in Europe in 2007 under the brand name of Brilliant peel (Fluoron/Geuder, Heidelberg, Germany).[306]

Bromophenol blue (BroB), also named tetrabromophenolsulfonephthalein, is a hydroxytriarylmethane color marker dye with a molecular weight of 670 daltons and the chemical formula of $C_{19}H_{10}Br_4O_5S$. BrB has been applied in laboratorial settings as an acid-base indicator as well as marker in gel electrophoresis.[279,330] The dark-blue dye may represent a novel and useful adjunct for both cataract and vitreoretinal surgery, although no commercial available product is yet available in the United States.

Patent blue (PB) is a hydrophilic anionic triarylmethane dye with the chemical formula of $C_{27}H_{31}N_2NaO_6S_2$ and a molecular weight of 582 daltons. The blue arylmethane dye prepared in sodium or calcium salt is applied as a fluorescent indicator to identify fungi in vitro.[348] It has also found use in localizing lymph nodes during oncologic surgery.[19,46] PB has been has been certified in Europe since 2003 for capsule staining during cataract surgery in a concentration of 0.24% under the brand name of Blueron (Geuder) and has been applied off-label during vitreoretinal surgery.[124]

CYANINE DYES

Cyanine dyes contain a -CH = group linking two heterocyclic rings containing nitrogen. Cyanine dyes are part of a larger group called polymethine dyes. Cyanine dyes are highly colored, organic com-