The Use of Trypan Blue Dye for Strabismus Reoperations, Surgery Complications, and Especially for the Identification and Recovery of a “Slipped” or “Lost” Extraocular Muscle

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ABSTRACT: Purpose: To evaluate trypan blue dye in strabismus surgery for tissue identification, to find the lowest optimal concentration, and to describe histological findings in tissue so stained.

Methods: Trypan Blue dye 0.1% was serially diluted and tested by tissue staining at progressively different concentrations. Fifteen patients were studied using the dye.

Results: Trypan Blue 0.1% was the optimal concentration. Muscle, tendon and fibrotic tissues were easily identified and distinguished with the dye at this concentration. Such tissue identification was most useful and enhanced the performance of strabismus surgical operations, and especially in identifying and retrieving “slipped” or temporarily “lost” extraocular muscles.

Also a thin basement-like membrane surrounding the superior oblique muscle tendons was disclosed with PAS stain, suggesting the presence of glycosaminoglycans.

Conclusions: Trypan Blue 0.1% contrasts the different tissues, dying the muscle sheath, tendons and fibrotic tissues, but not staining the sclera and muscle fibers per se.

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INTRODUCTION

Identification of muscle fibers and tendons in reoperated muscles, or slipped muscles can be difficult, if tissues are not clearly differentiated and distinguished.

Vital dyes are a great aid for that purpose. They can be intravital, applied to living organisms; or supravital, applied to living cells or tissues freshly removed from the body. Intravital stains are used in different types of ophthalmic surgeries—cataract, corneal and retinal—for visualization of the internal limiting and basal membrane. (1)

Vital dyes are classified according to their chemical composition. There are xanthene dyes (fluorescein sodium), cyanine dyes (indocianine green and infracyanine green), steroid dyes (triamcinolone acetonide acetate and flucinolone acetonide acetate), natural stains (alizarin red), arylmethane dyes (brilliant or acid blue, gentian violet, bromophenol blue and patent blue), thiazine dyesn (methylene blue), and azo dyes (trypan blue and Janus green).

Azo dyes contain nitrogen in the azo form—N5N—in their molecular structures. Sixty percent of all synthetic dyes are azo types. Trypan Blue (TB) is an azo dye, which gives bright, high intensity color, without toxicity, and its biggest advantage is cost-effectiveness. It is used at different concentrations: for cataract capsulorrhexis and pediatric cataract 0.1% and mixed with hyaluronate at 4%--; for keratoplasty 0.02%-, and for chromovitrectomy 0.06% to 0.2%. (2-4)

As TB has already been used for superior oblique tendon identification, we decided to study the use of this dye in extraocular muscular surgery, where tissues were hard to identify. (5)

The aims of this study are to evaluate the use of TB in strabismus surgery as an enhancer of tissue identification, to find the lowest optimal concentration of TB to identify specific tissues in strabismus surgery, to determine which tissues are dyed by TB, and to describe histological findings.

PATIENTS AND METHODS

All studies were performed with the informed oral consent of the subjects, conformed with the Declaration of Helsinki.

Inclusion criteria were patients with strabismus, who needed reoperation or were suspected to have muscle anomalies. Fifteen patients underwent strabismus surgery using Trypan Blue stain for tissue identification. All surgeries were performed by the same surgeon (FS). (See TABLE, on page XXXX.)

TB was diluted with balanced salt solution (BSS®) and tested at progressive different concentrations—100% of the product (TB 0.1%), 50%, 25%, and 12.5%. Iconographies at different dilutions were made, intraoperatively before and after the use of TB.

Tissues were stained with a cotton swab, soaked in TB, after dissecting the conjunctiva and the Tenon capsule. The use of a cotton swab to apply the dye avoids the spreading of the stain, which gives greater control when dying the area of interest. Balanced salt solution was irrigated to clean up the area. This procedure was repeated with decreasing concentrations of TB. The optimal staining was at 100% concentration (TB 0.1%).

We observed that TB 0.1% stained the muscular sheath, muscular tendon and fibrotic tissues, but it did not stain the sclera and muscle fibers. This enhanced tissue identification. In cases of strong scar tissues,
the staining process was repeated during the dissection process. This allowed progressive identification of the muscle sheath, simplifying the surgery.

Specimens obtained from the excision of tissues during surgery were processed by light microscopy. Sections from unfixed tissue were obtained with a cryostate, mounted in gelatin and examined unstained. Samples from muscle, tendons and fibrous scar tissue were fixed in 4% formaldehyde, embedded in paraffin and stained with hematoxilina and eosin, periodic acid Schiff (PAS), and Masson trichrome stain.

Out of fifteen patients we chose the first three cases to demonstrate in illustrations the stained area as follows:

Case 1

Patient with Apert syndrome suspected to have extra ocular muscle anomalies. TB was used to identify the superior oblique muscles. TB stained the left superior oblique fibers, and made it easy to hook the muscle. (See Figure 1 A,B,C, below)

Case 2

Patient with previous strabismus surgery suspected to have lost muscle. The tissues that were believed to be muscle were engaged by an eye muscle hook, isolated, and TB was applied dying the eye muscle tendon and the muscle sheath. We saw that the lateral rectus fibers were slipped, but not lost. (See Figure 2 A,B,C, top, next page)

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**Figure 1 A (Shokida et al):** Apert syndrome with agenesia of the superior oblique muscle. Absence of staining. **B and C.** Normal dying of the superior oblique tendon.
Case 3

**Figure 2 A and B (Shokida et al):** A: Staining of muscle sheath and intermuscular membrane (white arrow, in B) in a case of a “slipped” extraocular muscle. C. Staining of fibrotic tissues between the Tenon's capsule (between white arrows) and muscle sheath (black arrows) in repair by advancement of the remaining muscle fibers and its sheath.

Patient with three previous surgeries showed restricted strabismus. TB was applied for identification of fibrotic tissues. (See Figure 3 A,B,C, below.)

**Figure 3 A (Shokida et al):** Trypan blue staining of muscle sheath and fibrotic tissues of a previously operated muscle. B. Fibrotic scars (black arrows) dyed with trypan blue. C. Stained muscle sheath (white arrow) and muscle fibers (black arrow) which are not stained with trypan blue.
### Table

<table>
<thead>
<tr>
<th>Case</th>
<th>Strabismus Type</th>
<th>Findings</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Apert syndrome. First surgery.</td>
<td>Superior oblique muscle agenesis.</td>
</tr>
<tr>
<td>2</td>
<td>Lost muscle suspected. Two previous surgeries.</td>
<td>Slipped muscle by identification of the muscular sheath.</td>
</tr>
<tr>
<td>3</td>
<td>Restrictive strabismus. Three previous.</td>
<td>TB enhanced identification of tissues.</td>
</tr>
<tr>
<td>4</td>
<td>A pattern reoperation.</td>
<td>Superior oblique muscle tenectomy with TB stain.</td>
</tr>
<tr>
<td>5</td>
<td>Esotropia. Two previous surgeries.</td>
<td>DVD. One previous surgery. Adherence between recessed superior rectus and superior oblique muscle. AO SR recessed Scars AO re recessed medial rectus.</td>
</tr>
<tr>
<td>6</td>
<td>Consecutive exotropia. One previous surgery.</td>
<td>Fibrotic tissues in medial rectus.</td>
</tr>
<tr>
<td>7</td>
<td>Duane syndrome. First surgery.</td>
<td>Medial rectus anomalies with two fibrotic feet in the Eso Duane eye.</td>
</tr>
<tr>
<td>8</td>
<td>Hypocorrected exotropia. One previous surgery.</td>
<td>Short and fibrotic lateral rectus scars.</td>
</tr>
<tr>
<td>9</td>
<td>III nerve palsy reoperation.</td>
<td>Fibrotic tissues restrictive muscles.</td>
</tr>
<tr>
<td>10</td>
<td>Consecutive exotropia A pattern. Two previous surgeries.</td>
<td>Reoperated superior oblique muscle was difficult to identify.</td>
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<tr>
<td>11</td>
<td>Consecutive exotropia. Two previous surgeries.</td>
<td>Slipped medial rectus.</td>
</tr>
<tr>
<td>12</td>
<td>Exotropia reoperation.</td>
<td>Scars in the medial rectus muscle.</td>
</tr>
<tr>
<td>13</td>
<td>Esotropia. Three previous surgeries.</td>
<td>Adherences and fibrotic tissues.</td>
</tr>
<tr>
<td>14</td>
<td>Esotropia. One previous surgery.</td>
<td>Adherences and fibrotic tissues.</td>
</tr>
<tr>
<td>15</td>
<td>Exotropia. One previous surgery.</td>
<td>Adherences and fibrotic tissues.</td>
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</table>
RESULTS

We found that TB 0.1% is the optimal concentration to stain the tissues of and around the extraocular muscles. Muscle sheath, tendon and fibrotic tissues are easily identified with TB. It does not stain the sclera and muscle fibers, per se.

The dye did not persist longer than 24 hours after surgery. No adverse reactions, such as post-surgery inflammation or persistent staining, were seen in the 10 patients studied.

Histological findings show that frozen sections failed to disclose the TB stain in excised tissues. Light microscopy examination of fixed tissues showed a delicate thin basement like membrane surrounding the superior oblique muscle tendons. This structure was disclosed with PAS stain suggesting the presence of glycosaminoglycans.

DISCUSSION

In spite of directions and traditions, and conventions to the contrary, the authors will start the Discussion by reviewing once again, and even yet more broadly than in the Introduction, even yet more about the background of this clinical situation... before proceeding to a proper Discussion of the very Results and the rendering of our conclusions and recommended applications regarding the scientific medical experience being reported here:

In 1967, Norn described the vital staining of the conjunctiva using TB 1% by watching the stain grains in the histiocytes situated round the vessels. He found a diffuse stain in dead or degenerate cells and phagocytosis. (6)

Vital dyes are widely used in cataract surgery to stain the anterior capsule. Use of TB in cataract surgery makes the capsulorrhexis simple and safe. (7) It is also effective in managing white cataracts. (8, 9)

Indocyanine green 0.5% (ICG) and TB 0.1% were used to stain the posterior capsule to enhance visualization during posterior continuous curvilinear capsulorrhexis. Both dyes successfully stained the posterior capsule. (10)

Healey and Crowston (11) used TB to stain antiproliferative agents in in vitro experiments.

Antimetabolites, such as mitomycin C and 5-fluorouracil, are used during trabeculectomy to inhibit postoperative scarring. (11) Franks also agrees that visualization of the antimetabolite can be facilitated using TB. (12)

Dyes are also used for vitreoretinal surgery to improve the visibility of preretinal membranes. (13) It has been shown that proliferative membranes contain fibrous structures that can be stained using TB. The inner limiting membrane can be stained using indocyanine green or infracyanine green. Ting et al treated epiretinal membrane using double staining with indocyanine green and TB. (14) Stalmans used double vital staining with TB and infracyanine green to remove the epiretinal pucker. Infracyanine green is less likely to induce toxic effects on the retinal cells than indocyanine green. (15)

TB mixed with isovolumetrically glucose 10% was also used for macular hole. (16)

Wylegała used TB at 0.02% for deep lamellar keratoplasty, (17) while Sharma used it at 0.06% to identify the complication of perforated Descemet membrane during deep lamellar keratoplasty. John found no side effect after two years follow up of endothelial keratoplasty using TB as the stain. (18) TB was used for endothelial evaluation in the cornea bank for identifying devitalized cells. (19)
Another use of this stain is for resection of conjunctival cysts. Kobayashi used a mixture of TB and sodium hyaluronate to enhance the visualization of the capsule. (20)

What is more, TB is useful for identifying extraocular muscle anomalies. They are frequent in craniofacial dysostosis, such as Apert syndrome. Cuttone reported agenesis of the superior rectus muscle. (21) Greenberg published the absence of multiple extraocular muscles in a patient with Pfieffer syndrome. (22)

Absence and hypoplasia of the lateral rectus muscle were found in a child with congenital esotropia and mental and physical retardation, associated with duplication of the chromosome segment 7q32----q34. C. (23)

On the other hand, dyes have been questioned on their toxicity levels. Evaluation of ocular toxicity of TB in rabbit eyes showed that there were no signs of toxicity in the vitreous cavity at a concentration of 0.06%, but at 0.2% there was damage of the photoreceptors. (26)

Heilweil found no significant reduction of ERG responses, nor histological effects in the retina exposed to intravitreal TB at 0.06% in rabbits. However, it showed a disorganization of the retinal cells and there was ERG deficit at a higher concentration as 0.15%. (9)

Human corneal cells were used in in vitro experiments to quantify the toxicity of TB at different concentrations and exposure time. It was toxic to corneal endothelium and corneal fibroblasts at 0.1% concentration and exposure times more than 30 minutes. (27)

**DISCUSSION of RESULTS**

Although muscle anomalies can be identified with orbital MRI, TB is a useful aid for strabismus surgery. As we presented in Case 1, the patient with Apert syndrome had superior oblique unilateral agenesis, which was easily identified using the stain.

In Case 2, TB was useful for identifying a “slipped” muscle, in a case where we believed and feared that there was a “lost” extraocular muscle. The clinical features of a “slipped” muscle may resemble a “lost” muscle. (24)

There are many diagnostic maneuvers in identifying and finding a “lost” muscle, such as saccadic velocity, forced ductions, active force generation, orbital MRI, and the oculocardiac reflex. However, Lenart and Lambert said that "no single diagnostic test provides absolute reliability for determining [i.e., finding] a lost muscle.” (25) We found that the use of TB was a useful tool for recognizing the muscular capsule in “slipped” muscle cases.

**CONCLUSIONS**

As in our studies we did not find any adverse reactions, we conclude that Trypan Blue 0.1% is the optimal concentration to contrast the different tissues in strabismus surgery, because it dyes the muscle sheath, tendons and fibrotic tissues, but it does not stain the sclera and muscle fibers. It is a useful tool for reoperation in strabismus surgery.

**REFERENCES**

To the Authors

We presume that, in paragraph 3 of the Introduction, you did not mean a mathematical minus sign (-) in front of the various quoted TB concentrations and removed them to avoid that mis-interpretation.

Also: re quotes on “slipped” and “lost” muscles: these are not technically proper terms for these complications of surgery. The editor plans comments in his editorial “in this issue” which will explain this.
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FIGURES

Fig 1

Fig 2

Fig 3