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Experimental studies with *Staphylococcus aureus* in M-K media.

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This study reviewed the potential for survival of a pathogenic bacteria when inoculated into McCarey-Kaufman modified tissue culture media 199. A clinically isolated specimen of Staphylococcus aureus *was selected and rabbit eyes inoculated with 0.1 ml. of the organism in a suspension of 1 to 3 × 10⁻⁵. Upon enucleation 12 hours later no clinical signs of infection were noted. Limbal-conjunctival cultures were obtained on all eyes before and after application of the antibiotic. Corneas were stored in M-K media with standard penicillin-streptomycin added and cultured up to 48 hours. Our studies showed some recovery of the infecting organism from the media with topical antibiotic application but none following complete immersion of eyes in antibiotic. Direct cultures from corneal buttons taken 48 to 72 hours after antibiotic application by either method showed 1 to 3 colonies per plate in 25 per cent of eyes. In this study improperly applied antibiotic allowed some survival of* S. aureus *in the media.*

Rapid acceptance of McCarey-Kaufman modified[1, 2] tissue culture media 199 used for short-term corneal cold storage has come about with little supporting evidence for proper sterilization procedures. The media supplier (Warner Lambert Research Institute, Morris Plains, N. J.) guarantees sterility of the solution and container but donor corneas with known bacterial flora[3, 4] may serve as potential media contaminants. Treatment of donor eyes prior to grafting with antibiotic solutions has proved effective in reducing corneal and conjunctival organisms (Table I).[3-5] The purpose of this study was to determine whether M-K modifications are sufficient to suppress survival of donor eye pathogens introduced when corneal-scleral buttons are placed into the media.

Table I. Preferred methods of sterilizing donor eyes

Authors	*Method*
Doctor and Hughes[5]	10 minute immersion in Neosporin followed by 2 minute saline rinse.
Rollins and Stocker[3]	5 minute rinse with 10 c.c.'s Neosporin.
Polack, Locatcher-Khorazo, and Gutierrez[4]	2 minute drip of 0.5 c.c.'s Polymyxin B_1, Neomycin, and Gramicidin.

Penicillin-resistant *Staphylococcus aureus* was isolated from a patient with acute conjunctivitis and inoculated into rabbit eyes. Globes were enucleated 12 hours later, cultured, treated with neomycin-sulfate-Polymyxin B sulfate by either of two methods, recultured, and corneal-scleral buttons placed into M-K media for culture up to 48 hours.

Material and methods. Albino rabbits weighing between 1 and 2 kilograms were used. The lower cul-de-sac of each eye was inoculated with 0.1 c.c. of *S. aureus* in a suspension of 1 to 3 × 10^{-5} cells. The isolate was a penicillin-resistant strain obtained from a human eye with acute conjunctivitis. A 24-hour culture of the organism was suspended in Tryptose phosphate broth and plate dilutions were done to determine the concentration.

At 15 hours after inoculation the eyes were examined for clinical signs of infection, the rabbits sacrificed, and the enucleated eyes placed into sterile moist chambers kept at room temperature. Within 2 hours, all eyes were cultured prior to antibiotic treatment by swabbing 360° around the limbus using a moistened cotton tip applicator.

Two methods of antibiotic application were employed. Group A consisted of 25 eyes to which 20 drops of Polyspectrin (Neomycin sulfate polymyxin B sulfate) were placed directly onto the cornea. Fifty-four eyes in group B were irrigated with 20 c.c.'s of sterile normal saline then individually immersed in Polyspectrin for three minutes. All eyes were kept in sterile glass chambers at room temperature until cultures were obtained 30 minutes after antibiotic treatment.

Scleral-corneal buttons were then removed and placed in the McCarey-Kaufman media containing

Table II. Bacterial isolates from limbal swabs

	Group A (drops) 20 eyes (%)		*Group B (immersion) 34 eyes (%)*	
Pre-antibiotic cultures	*Staph. coag.* + heavy to moderate growth	100	*Staph. coag.* + heavy to moderate growth	100
	Neisseria Sp.	20	*Neisseria Sp.*	7
	Staph. coag. (−)	24	*Staph. coag.* (−)	15
	Proprionibacterium acnes	8	*Alpha-strep*	7
			Beta-strep (not group A)	8
			Proprionibacterium acnes	7
Post-antibiotic cultures	*Staph. coag.* + heavy to moderate growth	60	*Staph. coag.* + few	100
	Few	40	*Beta-strep* (not group A)	5

Table III. Cultures of *S. aureus* (Coagulase +) from TC-199 media

	12 hours	*24 hours*	*48 hours*
Group A (drops) 25 eyes	No growth 20 (80%)	No growth 25 (100%)	No growth 22 (88%)
	Staph. few (1-3 col) 5 (20%)		Staph. few (1-3 col) 3 (12%)
Group B (immersion) 54 eyes	No growth 54 (100%)	No growth 54 (100%)	No growth 54 (100%)

125 units of penicillin and streptomycin per milliliter (Microbiological Associates). The M-K media was stored at 4° C. and cultures were obtained at 12, 24, and 48 hours by placing a sterile cotton tip applicator near the bottom of the vial. Direct tissue cultures of 20 corneas from Group A (Polyspectrin drops) and 10 corneas from Group B (Polyspectrin immersion) were obtained by gently swabbing the corneal buttons in the media at 48 hours. On 20 corneas from Group B, buttons were punched out using standard techniques[6] and the endothelium streaked across and left on blood agar plates for incubation. For comparison, 10 globes were stored in standard moist chambers at 4° C. treated immediately by the Polyspectrin drop method and limbal cultures taken up to 48 hours.

All cultures were set up on blood agar plates and in thioglycollate broth. Subcultures were done on the thios at 48 hours. All cultures were incubated at 37° C. and examined for growth for five days. Standard methods of microbiological identification were employed and sterile technique was used throughout the study.

Results. At the time of enucleation, external examination of eyes including lid margins and adenexa showed no clinical signs of infection. Conjunctival limbal cultures taken at this time (12 to 15 hours after inoculation) consistently yielded the organism in moderate to heavy growth (Table II) whereas samples obtained later did not. The human donor eye situation was simulated when nonpathogenic bacteria as well as our inoculated organism were recovered.

Limbal swabs taken 30 minutes after antibiotic application by either the drop or rinsing-immersion technique yielded coagulase positive *S. aureus* in 100 per cent of eyes. Growth of the organism however was quantitatively reduced in Polyspectrin-immersed eyes (100 per cent few to minimal) compared to only 40 per cent few to minimal and 60 per cent moderate to heavy in Polyspectrin-drop eyes.*

Media cultures up to 48 hours (Table III) showed 100 per cent no growth in 54 Group B-immersed eyes. In comparison, 5 of 25 (20 per cent) Group A (drop) eyes at 12 hours and 3 of 20 (12 per cent) at 48 hours grew 1 to 3 colonies of *S. aureus* on blood agar plates.

Table IV outlines the 20 to 25 per cent incidence of corneal button cultures remaining positive after 48 to 72 hours of media storage.

Discussion.

Antibiotic application. Rollins and Stocker[3] and Polack, Locatcher-Khorazo, and Gutierrez[4] have shown high rates of recovery for a variety both pathogenic and nonpathogenic bacteria from human donor eyes obtained at autopsy. Cultures obtained by culturing only the cornea were less often positive than limbal-conjunctival-scleral swabs. *S. aureus* was the most frequently isolated pathogen in both studies.

A recent survey[8] of techniques employed by eye banks for donor eye sterilization showed that

*Standard Microbiological Procedures. The plates are streaked for isolation in 5 planes. Heavy growth: good amount of growth in all 5 planes. Moderate heavy: solid growth in first 3 planes and some growth into fourth and/or fifth plane. Moderate: solid growth in upper streak and some growth into second. Few to moderate: growth in upper primary streak. Few: 10 colonies or less.

Table IV. Direct cultures of corneal button*

	No. corneas cultured	*No. corneas positive for coag. + S. aureus*
Group A (drops):		
Swabs only	20	5 (25%)
Group B (immersion):		
Swabs	10	2 (20%)
Button on plate	20	4 (25%)

*After 48 to 72 hours in media.

67 per cent utilize antibiotic drop application. Comparison of two different methods for donor eye sterilization in this study showed a significantly greater effect using the rinsing-immersion technique (Group B) for both premedia and media cultures. The moderate and heavy growth of S. *aureus* noted in 60 per cent of globes cultured after Polyspectrin drops (Group A) and the 100 per cent no growth in media up to 48 hours in Polyspectrin (Group B) immersed eyes substantiates the work of Doctor and Hughes[5] and the recommendations of McCarey, Slappey, and Kaufman.[7] Rinsing plus immersing eyes for three minutes in Polyspectrin appears to be adequate for suppression of S. *aureus* in this model. We agree with the comments of Doctor and Hughes[5] who believe there is a beneficial effect of ridding the eye of bacteria by mechanical rinsing.

Media cultures. Recovery of S. *aureus* from media in Group A (drop) eyes in this study (Table III) suggests that penicillin-streptomycin doses alone are insufficient to suppress growth of the organism in this animal model. Rinsing plus antibiotic immersion of donor globes plus penicillin-streptomycin as recommended by McCarey, Slappey, and Kaufman[7] *do* appear to be effective in suppressing donor organisms inoculated into the media. We are concerned about and have not yet studied the potential for Gram-negative organisms such as pseudomonas to persist in media.

Corneal button cultures. Direct swabs of corneal-scleral tissue resting in TC-199 media and 48-hour old media buttons placed directly onto blood agar plates yielded 1 to 3 colonies of S. *aureus* in approximately 20 per cent of eyes. Although attempts were made to bring only endothelium into contact with plates, folding of several buttons during inoculation did occur which exposed epithelium to the plate. We conclude that small numbers of organisms were able to survive on the corneal epithelium even after 48 hours in media. No data is available, regarding the persistence of bacteria on human corneas immediately prior to grafting in moist chamber-stored eyes. For sake of comparison using the same animal model 10 of 10 (100 per cent) moist chamber-stored Polyspectrin drop-treated rabbit eyes inoculated with S. *aureus* showed recovery of the organism 48 hours after 4° C. storage. The clinical significance of these findings is unknown.

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Key words: M-K media, donor eyes, limbal, cultures, antibiotic, immersion, *Staphylococcus aureus.*

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Stereotaxic device for experimental eye surgery

JUAN ARENTSEN AND MARIO DURAN.

A stereotaxic device for experimental surgery in enucleated eyes is described. This instrument is made entirely of plastic, is inexpensive to make, and has proved to be an invaluable help in teaching microsurgical procedures to ophthalmic residents.

Most surgical procedures of the anterior segment of the eye are done today under the surgical microscope. Microsurgery, as it is commonly called, has improved results because with the magnification afforded by the microscope the surgeon can better appreciate the various surgical steps of his procedure. Training of eye surgeons demands extensive practice in experimental animals and enucleated eyes, particularly when using the surgical microscope. One of the more frequent