

DETERMINATION OF THE OCULAR FLORA
OF SOFT CONTACT LENS WEARERS

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its impact on the ocular flora. Below are the results of these investigations:²

1. There is no apparent difference of bacterial flora between healthy eyes of males versus females.
2. There is no seasonal (month by month) change of ocular flora.
3. The ocular flora between right eye versus left eye was almost always identical.
4. The ocular flora from cities around the world are essentially the same.

When a contact lens is applied to the cornea, many physiological changes seem to occur. Mandell demonstrated that even with a properly fitted contact lens a minimum of 2% corneal edema occurs.³ Thoft and Friend confirmed previous investigations that glycogen levels of the corneal epithelium decrease subsequent to contact lens wear.⁴ With increased utilization of corneal glycogen, tear film pH decreases due to metabolites of the Embden-Meyerhoff pathway and citric acid cycle. Hill and Leighton demonstrated that the corneal temperature remains unchanged with contact lens wear.⁵

²Ibid., p. 12.

³Mandell, R. B. and Polse, K. A. "Corneal Thickness Changes as a Contact Lens Fitting Index - Experimental Results and a Proposed Model." American Journal of Optometry. 46, p. 479.

⁴Thoft, R. A. and Friend, J. "Biochemical Aspects of Contact Lens Wear," American Journal of Ophthalmology. 80(1), p. 139-145, July 79.

⁵Hill, R. M. and Leighton, A. J. "Temperature Changes of Human Cornea and Tears Under a Contact Lens. I. The Relaxed Open Eye and the Natural and Forced Closed Eye Conditions," American Journal of Optometry. 42, p. 9.

The purpose of this project is to determine if any variance of ocular flora from the general population occurs with soft contact lens wear.

MATERIALS

The materials necessary for this project are listed below:

Sterile cotton swabs

0.9% unpreserved sterile saline

forceps

platinum inoculating loop

microscope slides

immersion oil

petri dishes

test tubes

wax pencil

autoclave

incubator (37°C)

optochin discs

media:

sheep blood agar

mannitol salt agar

bile esculin agar

gram stain:

crystal violet

iodine

ethyl alcohol - 95%

safranin

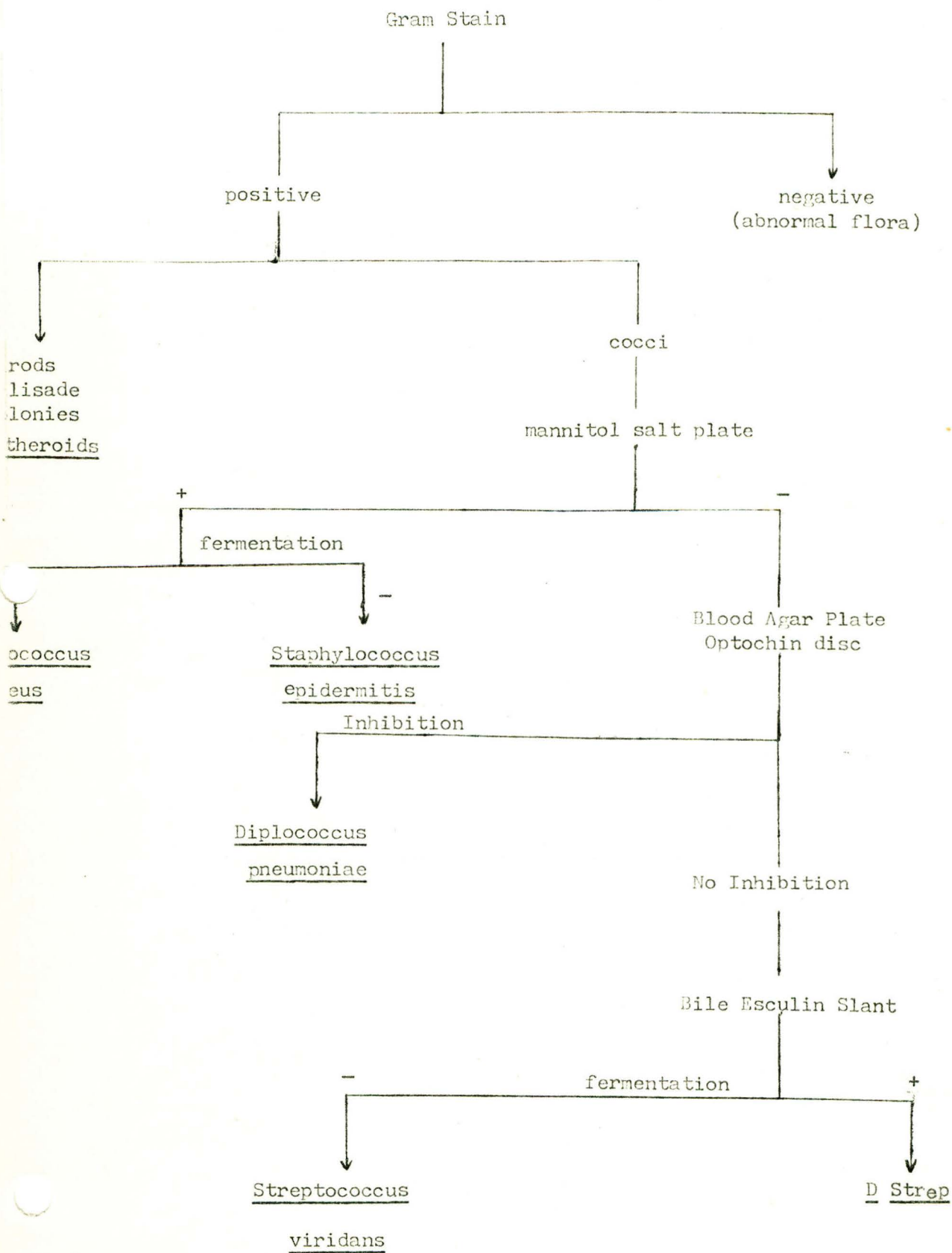
alcohol burner

PROCEDURE:

Eye cultures were obtained from soft contact lens wearers subsequent to contact lens evaluation and dispensing at the Ferris State College of Optometry Clinic. The volunteers for this study had been wearing soft contact lens for period of two weeks to 18 months. Eye cultures were obtained from wearers any time after two hours of wear for that particular day.

In order to obtain an accurate sample of ocular flora, aseptic techniques were mandatory. Sterile cotton swabs moistened with daily prepared and sterilized 0.9% saline were used to obtain the floral sample. Subsequent to removing the contact lens and prior to any installation of fluorescein, the patient was directed to gaze superiorly. The cotton swab was passed once along the lower cul de sac and immediately smeared onto a sheep blood agar plate. The culture plate was then inverted and placed into an incubator set at 37°C. The plate was examined twenty-four and forty-eight hours after inoculation to check for bacterial colonies. By examining the gross morphology, gram stain, and by selectively inoculating different types of media from colonies found on the smear plate, identification of the microorganisms were made. The following is a flow diagram and brief discussion of these procedures:

FLOW DIAGRAM



GRAM STAINING PROCEDURE

1. Pass a platinum inoculating loop through a flame until the wire becomes red hot to insure sterility.
2. Allow the loop to cool for one or two seconds and then obtain a small loopful of sample from the colony to be analyzed.
3. Smear the sample onto a clean glass slide and allow to dry.
4. Pass the dried slide through a flame in order to heat-fix the material onto the slide.
5. Stain the smear with prepared crystal violet for one minute.
6. Wash briefly with tap water.
7. Apply iodine solution to the smear and allow to stand for one minute.
8. Wash briefly with tap water.
9. Decolorize the smear with 95% ethyl alcohol until all excess stain is removed.
10. Counterstain smear with safranin for ten seconds.
11. Examine the prepared slide with a microscope.

MATERIALS USED FOR DIFFERENTIAL DIAGNOSIS:Sheep Blood Agar

This media contains infusion from sheep heart, tryptose, sodium chloride, and Bacto agar. Most microorganisms grow on this media. Partial and complete hemolysis can be observed by certain strains of streptococci and Staphylococci.

Mannitol Salt Agar

This media contains beef extract, proteose peptone, sodium chloride, d-mannitol, Bacto agar, and phenol red.

Koch demonstrated that on solid media Staphylococci were not inhibited by a concentration of 7.5% sodium chloride. Thus, mannitol salt agar contains an approximated 8% sodium chloride which is used for isolation of Staphylococci.

Staphylococci can be further differentiated by utilization of d-mannitol and phenol red, a Ph indicator. It has been demonstrated that S. aureus, in the presence of oxygen, metabolizes d-mannitol to lactic acid and small amounts of carbon dioxide. This reduces the Ph of the media, causing the Ph indicator to turn red. S. epidermitis will not exhibit this type of activity.

Bile Esculin Agar

This media is used to determine an organism's ability to hydrolyze esculin in the presence of bile. Bile esculine contains ferric citrate which combines with the ^{products} produce of esculine hydrolysis, acetoin. The resulting compound turns the media to a dark brown or black color.

Optochin Disc

Optochin discs (containing ethyl hydrocupreine hydrochloride) are used for differentiating Diplococcus pneumoniae from other alpha ^{hemolytic} hemolytic Streptococci.

This test is performed by streaking a SBAP with an inoculum of suspected organism. Place a disc onto the streaked media with sterilized forceps and incubate for twenty-four hours. A zone of inhibition indicates a positive test and is indicative of Diplococcus pneumoniae.

RESULTS:

Ocular smears from 55 soft contact lens wearers were cultured and differentiated by the previously described techniques.

Below are the results of my findings:

<u>Organism</u>	<u># Plates</u>	<u>Percentage</u>
<u>S. epidermitis</u>	23	42 %
<u>S. viridans</u>	15	27 %
<u>S. aureus</u>	8	14.5%
<u>D strep</u>	5	9.0%
<u>S. pneumoniae</u>	2	3.6%
No growth	19	34.5%

DISCUSSION:

Studies of the normal ocular flora by Khorazo and Thompson demonstrated that approximately 23% of their subjects appeared to have bacteria.⁶ Results from this study show an approximate 10% increase in subjects without ocular flora. This may be attributable to one of two possibilities. First, there may be an accumulation of bacteriostatic agents from preserved saline within the lens matrix. Another possibility for reduced flora may be due to endogenous exotoxins released from bacteria that are destroyed through the heat sterilization process of contact lenses.

⁶Khorazo, D. A. and Locatcher. Microbiology of the Eye, 1968, p. 28.

Even though S. aureus is classified as a facultative anaerobe, studies have demonstrated that optimum growth occurs under aerobic conditions.⁷ Thus, it would seem logical that with contact lens wear with subsequent reduced oxygen tension, the prevalence of S. aureus would drop. This does not, however, seem to be the case.

S. epidermitis is considered a nonpathogenic organism which is found mainly on the skin and mucous membranes of individuals.⁸ By handling the contact lenses during normal insertion and removal techniques, one would expect to find a greater percentage of cultures containing S. epidermitis. This, however, does not seem to be the case. In fact, it is apparent that the number of cultures containing S. epidermitis is reduced by 19%. This may be secondarily due to the increased percentage of S. aureus which is known to produce a powerful bacteriostatic exotoxin.⁹

The reason for no evidence of Diphtheroids in my results can be attributed to experimental error. Diphtheroids do not grow on Sheep Blood Agar, but require a special media, Loeffler Blood Serum, which contains dextrose broth.

⁷ Bergeys Manual of Determinative Bacteriology, 1968, p. 28.

⁸ Ibid., p. 971

⁹ Ibid., p. 962.

As stated previously, glycogen levels of the corneal epithelium decrease subsequent to contact lens wear. Differences of opinion regarding the cause of this phenomena have resulted. Some investigators believe that the reduced storage of glycogen is secondary to anoxia. Hill, Augsburger, and Uniacke demonstrated that epithelial glycogen levels of silicone contact lens wearers are still only approximately 80% of normal.¹⁰ With a reduction of ocular flora, it seems plausible that the possibility of ocular flora reducing the epithelial glycogen levels secondary to corneal traumatization can be safely ruled out.

It is apparent from these results that the normal ocular flora has changed with soft contact lens wear and may warrant further investigation. Also, in order to obtain a more accurate assessment of these changes, a larger sample is required.

¹⁰Hill, R. M., Augsburger, A. R., and Uniacke, C. A.: Oxygen permeable "hard" contact lenses. Contact Lens 3:40, 1972.

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