Anaerobic bacterial endophthalmitis was studied in rabbits following intravitreal injection of live Fusobacterium necrophorum. Clinical response, bacterial recovery, and histopathology were studied. An inoculum of approximately 50 organisms produced endophthalmitis in 59% of injected eyes, while 1000 or more organisms produced endophthalmitis in 100% of injected eyes. The course and severity of disease seemed to be independent of the concentration of bacteria above a minimal inoculum size. Affected eyes showed progressive endophthalmitis. Histopathologic changes corresponded to the clinical gradation of endophthalmitis, including progressive retinal necrosis. Invest Ophthalmol Vis Sci 27: 115-118, 1986

Endophthalmitis is a catastrophic complication of intraocular surgery, penetrating injury, and endogenous infection. The etiologic agent in many cases is unclear and routine aerobic bacterial cultures are often negative. Only recently has there been any emphasis on the role of anaerobic bacteria in endophthalmitis. Since anaerobic bacteria are present in some normal as well as infected conjunctivae, anaerobic bacterial infection should be considered in cases of endophthalmitis.

In this report, we describe the production of anaerobic bacterial endophthalmitis following intravitreal injection of live Fusobacterium necrophorum in rabbits. After preliminary studies to determine a clinical grading system and an optimal concentration of inoculum, eyes were injected and studied for clinical progression of disease, bacterial recovery and histopathology. Clinical and histopathologic features of experimental anaerobic endophthalmitis were characterized.

Materials and Methods. Clinical isolates of F. necrophorum from the blood of patients with septicemia were stored at −70°C in skim milk and used later for intravitreal injection. F. necrophorum was selected because it is a strict anaerobe and a virulent pathogen. While it would be expected to produce clinical disease, it would also serve as a stringent test of our technique for the preparation and recovery of anaerobic bacteria. The isolates were thawed and then subcultured onto Brucella blood agar at least twice prior to inoculation into thioglycolate broth for 24-48 hr. The broth was diluted in sterile, pyrogen-free, non-bacteriostatic buffer (.15 M NaCl; .02 M phosphate buffer) to a turbidity corresponding to a number 1 MacFarland standard [10^8 colony forming units (CFU)/ml]. Aliquots of serial dilutions made in the sterile buffer were used for both the determination of viable counts (rotator-pipet method) and intravitreal injection. The injection aliquots were transplanted in Hungate style screw cap tubes containing an oxygen-free atmosphere and glass beads (to facilitate mixing).

Male, New Zealand albino rabbits (5-5.5 lbs) were obtained from ABC Laboratories (Pomona, CA). Our investigations utilizing animals conformed to the ARVO Resolution on the Use of Animals in Research. For intravitreal injections, animals were anesthetized with intramuscular injections of Xylazine HCl (3 mg/kg) and ketamine HCl (15 mg/kg). Corneas were anesthetized with topical proparacaine HCl (0.5%). For vitreal aspirates and enucleations, animals were euthanized with sodium pentobarbital (6 gr/ml). To prevent loss of inoculum, intraocular pressure was reduced by removal of 0.1 ml aqueous humor. Intravitreal injections were through the pars plana, using a 30-gauge needle on a tuberculin syringe, and ranged from 5X10^7 to 1X10^7 bacteria per 0.1 ml per eye. Control eyes were injected with either 0.1 ml saline or thioglycolate broth diluted 1:4 in buffer. Ocular examinations were performed daily using an indirect ophthalmoscope and a Haag Streit slit lamp and the eyes graded according to Table 1. As endophthalmitis progressed, animals were randomly selected at each grade for vitreous aspirates and enucleation. Enucleated eyes were partially opened at the equator and fixed in 10% neutral buffered formalin. After 48 hr, the globe was bisected around the equator and one half of the posterior hemisphere paraffin embedded, sectioned, and stained with hematoxylin and eosin (H & E). The vitreal aspirates were transported in Port-a-cul vials (Baltimore Biological Laboratory; McConkeyville, MD.). After vigorous vortexing, each was subcultured in an anaerobic
Clinical onset of disease

other bacterial species.

attempt to recover bacteria.

when an aspirate was collected, it was cultured both for

were negative after 7 days, then the thioglycolate broth,

was also inoculated from the Port-a-cul vial, was

subcultured onto fresh BA and CA plates as a final

chromatography to detect the conversion of threonine

Eyes developing endophthalmitis

Histopathology: Eyes graded clinically as 1+

endophthalmitis had an intact retina with a minimum

exudation and polymorphonuclear cells in the vit-

fibrinous reaction and retinal vessel dilatation were

identified with 100% accuracy by indirect ophthal-

microscopic examination. Moderate vitreous haze, and

fibrinous reaction and retinal vessel dilatation were

noted with progression to 4+ endophthalmitis. By post-injection day 2, eyes that eventually progressed to endophthalmitis could be identified with 100% accuracy by indirect ophthalmoscopic examination. Moderate vitreous haze, and fibrinous reaction and retinal vessel dilatation were noted with progression to 4+ vitreous haze by day 3–4. In eyes injected with as few as 50 organisms, vitreous signs were slower to develop, with some animals developing 1+ vitreous signs as late as 4–5 days after injection. However, once vitreous signs did appear, 100% of eyes that were followed progressed to 4+ endophthalmitis. Other associated clinical signs in some animals were lid edema and chemosis.

F. necrophorum was the only organism isolated from test eyes. No contaminants were noted (Table 2).

Histopathology: Eyes graded clinically as 1+ endophthalmitis had an intact retina with a minimum of exudation and polymorphonuclear cells in the vitreous cavity (Fig. 1). Eyes which were 2–3+ showed

chamber onto Brucella blood agar (BA) and chocolate agar (CA) plates and into a thioglycolate broth. The plates were incubated in an anaerobic (BA) or CO₂-enriched (CA) atmosphere for at least 48 hr. The growth of aerobic or facultative anaerobic bacteria was noted and a gram stain performed. All colony types growing on the anaerobic plates were processed according to standard methods. The identification of Fusobacterium necrophorum was confirmed using gas-liquid chromatography to detect the conversion of threonine and lactate to propionate. If the BA and CA plates

1+ Few aqueous cells (<10–20 cells per high power field), minimal flare.

Slight haze in vitreous (retinal vessels clearly seen)

2+ Moderate anterior chamber reaction (>20 cells, flare)

Progressive haze in vitreous (decrease in clarity of retinal details)

3+ Poor circulation of aqueous cells, early fibrin strand formation, dense cellular reaction

Obscuration of retinal details; vitreous strands and clumps

4+ Marked fibrin deposits in anterior chamber, synchiae, hypopyon

Unable to see retina; white reflex: large clumps of vitreous exudates

Table 1. Clinical grading system endophthalmitis

Table 2. Intravitreal injection of F. necrophorum endophthalmitis/bacterial isolation

Results. Initially, infected and control eyes of all animals exhibited a mild transitory anterior segment reaction consisting of variable iris and perilimbal vascular injection, and some aqueous cells with flare. By 3 days post-injection, the control eyes exhibited minimal to no inflammation. The concentration of F. necrophorum producing endophthalmitis in greater than 50% of animals was determined to be between 5.0 × 10⁴ and 3 × 10⁵ organisms per eye (Table 2). Although the percentage of rabbits developing endophthalmitis increased with higher concentrations of F. necrophorum, there was no increase in the ultimate intensity of disease. At ≥10³ organisms per eye, all eyes exhibiting vitreous involvement on day 2 progressed rapidly to 4+ endophthalmitis. By post-injection day 2, eyes that eventually progressed to endophthalmitis could be identified with 100% accuracy by indirect ophthalmoscopic examination. Moderate vitreous haze, and fibrinous reaction and retinal vessel dilatation were noted with progression to 4+ vitreous haze by day 3–4. In eyes injected with as few as 50 organisms, vitreous signs were slower to develop, with some animals developing 1+ vitreous signs as late as 4–5 days after injection. However, once vitreous signs did appear, 100% of eyes that were followed progressed to 4+ endophthalmitis. Other associated clinical signs in some animals were lid edema and chemosis.

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Unable to see retina; white reflex: large clumps of vitreous exudates

Table 1. Clinical grading system endophthalmitis

<table>
<thead>
<tr>
<th>Grade</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Clear media (anterior chamber, vitreous)</td>
</tr>
<tr>
<td>1+</td>
<td>Few aqueous cells (&lt;10–20 cells per high power field), minimal flare.</td>
</tr>
<tr>
<td></td>
<td>Slight haze in vitreous (retinal vessels clearly seen)</td>
</tr>
<tr>
<td>2+</td>
<td>Moderate anterior chamber reaction (&gt;20 cells, flare)</td>
</tr>
<tr>
<td></td>
<td>Progressive haze in vitreous (decrease in clarity of retinal details)</td>
</tr>
<tr>
<td>3+</td>
<td>Poor circulation of aqueous cells, early fibrin strand formation, dense cellular reaction</td>
</tr>
<tr>
<td></td>
<td>Obscuration of retinal details; vitreous strands and clumps</td>
</tr>
<tr>
<td>4+</td>
<td>Marked fibrin deposits in anterior chamber, synchiae, hypopyon</td>
</tr>
<tr>
<td></td>
<td>Unable to see retina; white reflex: large clumps of vitreous exudates</td>
</tr>
</tbody>
</table>

Table 2. Intravitreal injection of F. necrophorum endophthalmitis/bacterial isolation

<table>
<thead>
<tr>
<th>Organism per Eye</th>
<th>5 × 10⁴</th>
<th>3 × 10⁵</th>
<th>10³ to 10⁶</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes developing endophthalmitis</td>
<td>16/27*</td>
<td>4/10</td>
<td>13/13</td>
<td>33/50</td>
</tr>
<tr>
<td>Isolation of F. necrophorum from vitreous†</td>
<td>0/8‡</td>
<td>—</td>
<td>—</td>
<td>0/8</td>
</tr>
<tr>
<td>Grade 0§</td>
<td>0/3</td>
<td>—</td>
<td>—</td>
<td>0/3</td>
</tr>
<tr>
<td>Grade 1</td>
<td>0/5</td>
<td>—</td>
<td>—</td>
<td>0/5</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3/4</td>
<td>—</td>
<td>—</td>
<td>3/4</td>
</tr>
<tr>
<td>Grade 3</td>
<td>2/2</td>
<td>3/3</td>
<td>12/12</td>
<td>17/17</td>
</tr>
<tr>
<td>Grade 4</td>
<td>0/22</td>
<td>0/3</td>
<td>0/12</td>
<td>0/37</td>
</tr>
<tr>
<td>Isolation of other bacterial species from vitreous†</td>
<td>5</td>
<td>1</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Clinical onset of disease</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Day 1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>7</td>
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<tr>
<td>Day 3</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>Day 4</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
</tbody>
</table>

* Expressed as eyes positive for endophthalmitis over total eyes injected.
† Vitreal aspirates were not taken from every eye in the study due to either the unexpected death of the animal or the lack of clinical disease. However, when an aspirate was collected, it was cultured both for F. necrophorum and other bacterial species.
‡ Expressed as positive cultures over number of eyes sampled. For concentrations ≥3 × 10⁵ organisms per eye the progression to 4+ endophthalmitis occurred so rapidly (within 24 hours of onset of clinical signs in some cases) that aspirates were not collected at lesser grades.
§ Vitreal aspirates were collected on day 7 if no clinical signs were observed.
focal areas of retinal detachment, focal retinal necrosis, subretinal exudates, marked choroid vessel engorge-
ment, and moderate vitreous exudate with increasing numbers of polymorphonuclear cells. Eyes clinically
graded as 4+ endophthalmitis had focal to extensive retinal inflammation and necrosis and dense inflamm-
atory cell infiltrates in the vitreous and subretinal areas (Fig. 2).

Discussion. Anaerobic bacteria are commonly found
on human mucosal surfaces, including the conjunctivi-
vae,1,2 but have infrequently been reported in cases of conjunctivitis, orbital cellulitis, canaliculitis, dacryo-
cystitis, keratitis, and endophthalmitis.4-8 Due to the
fastidious nature of anaerobic bacteria and the special
requirements for transport and culture, studies of an-
aerobic ocular infections, particularly endophthalmitis,
have seldom been pursued.

In 1975, Forster4 reported a case of endophthalmitis
due to Propionibacterium acnes. Subsequently, in 1977,
Jones and Robinson5 reported 10 cases of anaerobic endophthalmitis following penetrating eye injury or
surgery. Cultures of intraocular fluid yielded Propioni-
bacterium, Bacteroides, Fusobacterium, Lactobacillus,
Veillonella, and Actinomyces species as well as aerobic
bacteria. Two of these cases were infected solely with
anaerobic organisms: Lactobacillus fermentus and P.
acnes. In 1978, Forster6 isolated P. acnes in 2 eyes
following intraocular lens insertion. Both eyes exhibited
a smoldering clinical inflammation with late onset,
emphasizing the fact that organisms of low virulence
are capable of eliciting endophthalmitis. Peyman et al7
reported in 1980 3 cases of anaerobic endophthalmitis
with P. acnes, a Clostridium species and a Peptostrep-
tococcus species as the single etiological agents. Other
than a few scattered reports there have been no exten-
sive studies on anaerobic bacterial endophthalmitis
and, to our knowledge, few, if any, experimental studies
have been reported in the English literature.

Using F. necrophorum, we have established an an-
imal model of anaerobic bacterial endophthalmitis that
has allowed us to study the progressive clinical stages
of the disease and to correlate these with the histo-
pathologic changes. Following F. necrophorum infec-
tion in the eye, there is a variable progression of in-
flammatory disease, depending on organism inoculum
concentration, with marked vitreous reaction, mem-
brane formation, and retinal necrosis. The inability to
isolate the organism at the lower grades of endophthal-
mitis may be due to small numbers of organisms in
the vitreous at the time of aspiration and reflect the
difficulties in identifying such ocular anaerobic infec-
tions, even by laboratories experienced in these tech-
niques.

In the study reported here, very small numbers of
F. necrophorum produced a rapidly progressive en-

Fig. 1. 1+ Endophthalmitis. Minimal exudate with a few poly-
morphonuclear inflammatory cells seen in the vitreous (V). Retina
(R) and sclera (S) are uninvolved. Choroid (Ch) exhibits a slight en-
gorgement of blood vessels (X100).

dophthalmitis. Other species (eg Propionibacterium
acnes, Peptostreptococcus magnus, Clostridium per-
fringens) need to be evaluated, as such organisms are
more commonly found as resident flora of the external
eye.9,10 Other aspects of anaerobic endophthalmitis,
such as the role of endotoxins, and other predisposing
factors like the effects of intraocular foreign bodies (in-
cluding intraocular lenses) and possible synergy be-
tween aerobic and anaerobic organisms in precipitating
intraocular infection, need further study. Quantitation

Fig. 2. 4+ Endophthalmitis. Vitreous (V) is filled with polymor-
phonuclear inflammatory cells and exudate. Retina (R) is detached
with sub-retinal inflammatory cells (curved arrow) and serous exudate.
Choroid (Ch) is distended with marked blood vessel engorgement.
Inflammatory cells can be seen throughout all tissue layers, including
the retina (straight arrow) (>100).
of bacteria from ocular aspirates will become an important aspect of future studies.

**Key words:** anaerobic bacteria, *Fusobacterium necrophorum*, experimental endophthalmitis, rabbits

**Acknowledgments.** The authors thank Ann Dawson, Ann Guild, and Ellen Narver for technical assistance in performing these studies and in the preparation of this manuscript.

From the Department of Ophthalmology, University of Southern California, and the Estelle Doheny Eye Foundation*, and Infectious Disease Section of VA Wadsworth Medical Center and Department of Medicine, UCLA,† Los Angeles, California. Supported in part by Research to Prevent Blindness, New York, New York. Submitted for publication: March 11, 1985. Reprint requests: Ronald E. Smith, MD, Department of Ophthalmology, Estelle Doheny Eye Foundation, 1355 San Pablo Street, Los Angeles, CA 90033.

**References**


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**Inexpensive Stereoscopic CCRG Camera for Lens/Cataract Photography In Vitro**

Leo T. Chylack, Jr. and William H. Tung

In 1978 the American Cooperative Cataract Research Group (CCRG) adopted a stereoscopic camera system for photographing human cataracts in vitro based upon a Zeiss OPMI I operating microscope (Carl Zeiss, Inc.; Oberkochen, West Germany). Photographs obtained with this system were used to classify human cataractous change according to the standardized CCRG protocol. Classification data correlated with laboratory data furthered the attempt to define the biochemical or biophysical basis for specific types of cataractous change. Presently, the high cost of this camera (exceeding $20,000) precludes its use by many laboratories wishing to do human lens research. This study describes an inexpensive (less than $2,200) alternative camera for this type of photography. Adjacent frames on the film strip constitute stereo pairs which can be viewed in a modified stereo viewer. In the original CCRG camera both members of a stereo pair were included in the same frame. The quality of the stereo images obtained with this new system nearly equals that with the original Zeiss system. It is hoped that this inexpensive system will allow more scientists to participate in CCRG-related research and increase the supply of intracapsularly extracted cataracts available to all collaborating CCRG scientists. Invest Ophthalmol Vis Sci 27:118-122, 1986

In 1978 Chylack published a system of cataract classification, and in 1980 it was adopted by the American Cooperative Cataract Research Group (CCRG). High quality, artifact-free, color, stereoscopic photographs were obtained with the system's modified OPMI I operating microscope with a Zeiss-Urban stereoscopic adaptor (Carl Zeiss, Inc.; Oberkochen, West Germany). The cataract classification format has been improved, simplified, and employed in studies of the associations among age, sex, nuclear color and opacity, and the structure and light scattering properties of human cataracts. Originally it was hoped that some of the in vivo cataract classification methods could be validated by postoperative photography and in vitro classification of intracapsularly extracted lenses. However, the decline in availability of intracapsular cataract extraction in the medical centers processing the original CCRG classification apparatus makes it unlikely that these same centers can complete such a study. The need for a light, inexpensive portable classification camera may facilitate the completion of such studies, either in the United States or elsewhere.

In spite of the acknowledged usefulness of the original CCRG photographic apparatus, its present high cost (more than $20,000) precludes its widespread use. It is the purpose of this study to describe a compara-