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Studies on Uveitis

Part II: Hypotheses with case reports

Philip C. Hessburg, MD*

A number of patients with and without uveitis are used to show that this study of aqueous microbiology suggests the following hypotheses:

1. Routine techniques currently used in most microbiology laboratories overlook classical and variant bacteria which might be isolated from clinical specimens with improved microbiological techniques.
2. Bacterial forms may be present in other ocular tissue when not present in the aqueous.
3. Polymicrobial infections probably occur rather frequently, especially in chronic or subacute inflammatory states.
4. Alterations in various host factors may subject some persons to a greater incidence of inflammatory episodes including uveitis.
5. Antibiotics used in a regimen similar to that advocated to reduce recurrences of chronic Staphylococcal or Streptococcal disease may ameliorate the pattern of uveitis in some patients, especially if such regimens take into account the cell wall defective forms of these organisms.
6. The eye in some uveitic situations may be as resistant to microbial eradication by chemotherapeutic or biologic mechanisms as is the kidney in glomerulonephritis or the bone in osteomyelitis.
7. Sarcoid and its attendant uveitis may be related to a variant Mycobacterium species.

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Hypotheses with Case Reports

Hypothesis 1

ROUTINE techniques currently used in most microbiology laboratories overlook classical and variant bacteria which might be isolated from clinical specimens with improved microbiological techniques.

Case 1

An aqueous specimen was obtained by anterior chamber tap (ACT) of the right eye of a 59-year-old white male diabetic with active postoperative uveitis. Forty-six days earlier, an intracapsular cataract extraction had been performed by another ophthalmologist. Postoperatively the patient was treated with a combination atropine-steroid drop daily. Two days before admission the patient noticed pain, decreased vision, and redness of the right eye, and he was referred with "probable endophthalmitis" of the right eye. He had never had an attack of uveitis. An ACT was performed on the day of admission, and treatment was begun immediately with systemic antibiotics, systemic steroids, topical cyclopegics, and topical antibiotics. A synthetic penicillin was given intravenously and gentamycin sulfate intramuscularly. These were continued for nine days, as was 60 mg of oral prednisone. The eye cleared rapidly. On discharge, 1+ cells and 1+ flare remained in the anterior chamber. A great deal of debris remained in the vitreous, and vision was correctable to 20/100.

The eye remained clear until one month after discharge, when the inflammation of the right eye became exacerbated. Again, systemic and local steroids were utilized. However, by this time bacterial cell wall defective bacterial forms (CWDF) had been isolated from the aqueous, which had been removed by ACT on the first admission. Therefore, the patient was placed on alternate week therapy with oral penicillin G 250 mg QID and oral erythromycin 250 mg QID, which were maintained for several months. The patient has had no further attacks in the last four years.

Slit-lamp examination at time of ACT: marked injection of the right eye with hypopyon and a poor red reflex. Four+ cells and 4+ flare and many fine KP were noted. Much debris was present in the vitreous, with a poor red reflex.

Serology: VDRL, Kolmer, and FTA absorption tests were nonreactive. Darkfield aqueous examination: no spirochetes were seen,

although many mononuclear WBCs were noted. Aqueous cytology: Leishman's stain of a smear of the aqueous revealed many neutrophils (Figure 4A) chained together by intercellular connections or cytoplasmic bridges. Degenerating neutrophils and an occasional basket cell were present, as were small lymphocytes and hypertrophying lymphocytes. Cocci were noted in the aqueous (Figure 4C), along with "transitional bacterial forms". Complete blood count: hemoglobin 14.0, WBC 3,500, neutrophils 77, lymphocytes 23. Antinuclear factor: negative.

Microbiology

During the third microbiological phase, aqueous was inoculated into tubes containing a variety of media (see Figure 2).^{*} A small bacterial CWDF, which on some smears was thought to be a tiny bacillus (Figure 7C), was recovered on four different media. These Dienes and acridine orange-positive colonies (Figures 4D, E) grew out on thioglycollate, Mattman's thioglycollate "X", Medill-O'Kane and Veal Heart Infusion broth. The latest of these media to be considered positive was observed and photographed on the 33rd day of incubation.

A specimen of blood removed at the time of the ACT was also cultured and a bacterial CWDF recovered from both the Tryptose phosphate broth (Figure 4F) and the Brucella broth. No classical organisms were recovered from the blood.

Comment

In this patient there was suspicion of bacteria from the gram stain of the aqueous. Much of the gram-negative material on this smear would be labeled "crud" (Figure 4B) by some technicians. In only a few places were there transitional forms sufficiently characteristic on morphological grounds to be interpreted as "cocci". These organisms did not grow out quickly or easily, however, and the aqueous was reported as showing "no growth" by routine microbiological studies. Prolonged incubation time with special media was necessary to yield microorganisms.

In another patient, an acutely ill young man, we studied aqueous, blood and cerebrospinal fluid, all of which were interpreted as showing "no growth" by the general microbiology laboratory. Using our specialized techniques, we recovered a peculiar *Actinomyces* from the spinal fluid. This organism was identified by Dr. R. Gordon of Rutgers University as an "atypical strain of *Nocardia dassonvillei*". In light of Wirostko and Johnson's recent isolations⁹⁸ of an *Actinomyces*-like agent from the aqueous of some patients with uveitis, Dr. Gordon's identification is particularly intriguing.

^{*} Figures 1-3 are also shown in Pt. I, Aqueous Studies, which appeared in the Winter, 1977 issue of the Journal.

Hypothesis 2

Bacterial forms may be present in other ocular tissue when not present in the aqueous.

Case 2

An aqueous specimen was obtained from an ACT performed on the right eye of an 82-year-old white man with active generalized uveitis who had had numerous earlier bilateral attacks of uveitis. The current attack had been in progress for over three months. He had had innumerable previous treatments with local and systemic steroids and with various combinations of local and systemic antibiotics.

The patient's right eye was blind, glaucomatous, and painful. Sixty-one days after the ACT an evisceration was performed, and a specimen of uvea obtained under sterile conditions, which was then minced and cultured.

Ophthalmological examination at time of ACT: the right eye showed no light perception, severe corneal edema, glaucoma, and rubeosis iridis. There were fine KP and 1+ flare, but no cells noted by slit-lamp examination through the semi-opaque cornea.

Serology: VDRL, RPCF, and FTA absorption tests all nonreactive. Darkfield aqueous examination: no spirochetes seen. Aqueous cytology: only red blood cells noted by Leishman's stain of the aqueous. Complete blood count: hemoglobin 14.8, WBC 7,000, neutrophils 53, eosinophil 1, lymphocytes 45, monocytes 1. Antinuclear factor: weakly positive, homogeneous reaction.

Microbiology

First phase microbiological studies of the aqueous remained negative for 62 days of incubation. The specimen of uveal tissue was minced under sterile conditions and handled like an aqueous specimen. On several occasions, a Chanock's agar plate, subcultured from initial thioglycollate inoculation media, was considered suspicious by microscopic study. The plate was opened after 48 days and an agar block pushed across a sterile slide. Dienes positive and acridine orange-positive colonies of a gram-negative branching bacterial CWDF were photographed.

Comment

It is probable that bacterial CWDF are present intracellularly when they are not present in the aqueous. In this patient bacterial forms were not recovered from the aqueous late in the course of an attack but were recovered from tissue.

This case also illustrates the difficulty of deriving statistically significant data. This patient must be included in the group of patients with generalized uveitis but without CWDF in the aqueous, since in this case the CWDF were recovered from tissue only.

The organism recovered from Case 2 in its cell wall defective form was found by Dr. L. Mattman of Wayne State University

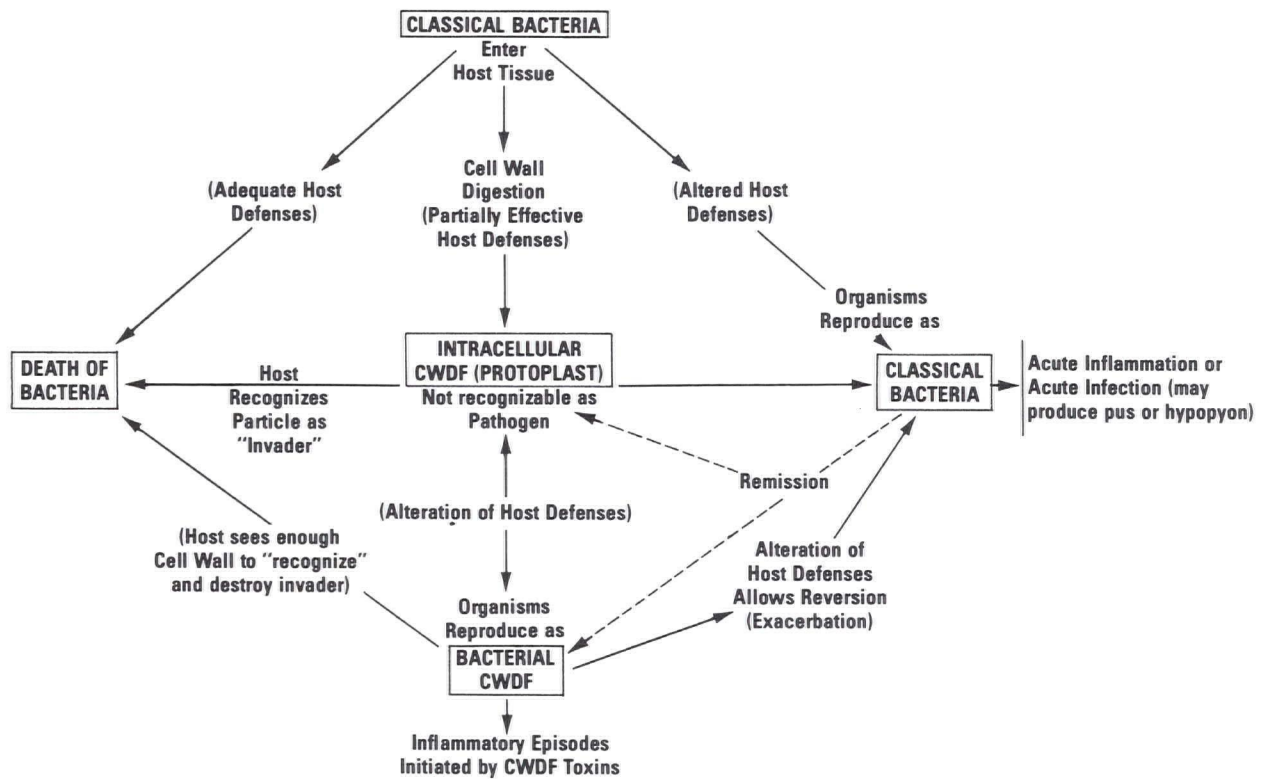


Fig. 1

Possible Pathogenic Pathways: Within the host a bacterium may be swiftly dispatched by phagocytosis and total digestion; may be phagocytosed but incompletely digested to become an intracellular CWDF; may reproduce either intra- or extracellularly as a bacterial CWDF or protoplast; or may reproduce as a classical bacteria. This diagram, which takes into account most of these possibilities, is adapted from the work of Sharp.³⁴ Inflammatory episodes either in response to bacterial forms or their toxins may occur with alterations in the host defenses in several ways.

to be nonpathogenic for mice; but when she inoculated it intraperitoneally in a rabbit prestressed with cortisone, it caused kidney abscesses from which she could recover only CWDF.

Another case was similar to this one in that the aqueous was negative, while organisms were recovered from tissue. This latter patient was a diabetic with bilateral chronic iridocyclitis. Two anterior chamber taps from the right eye and one from the left were completely nonproductive, but a specimen of vitreous removed following cataract extraction yielded a bacterial CWDF which finally reverted to *Staphylococcus epidermidis*.

Hypothesis 3

Polymicrobial infections probably occur rather frequently, especially in chronic or subacute inflammatory states.

Case 3

An aqueous specimen was obtained by anterior chamber tap (ACT) of the left eye of a healthy, white 42-year-old man who was not

known to have uveitis. While at work seven days earlier, the patient had been struck in the left eye by a penetrating ferrous foreign body, which was removed during an extracapsular cataract extraction. Anterior chamber tap immediately preceded this surgery. The postoperative course was one of low-grade inflammation with slow absorption of cortical remains. A postoperative pupillary membrane became increasingly dense over the next three years, eventually requiring a discission. The visual acuity was 20/25+3. Systemic erythromycin and trisulfapyrimidines were used before and after both surgical procedures. The patient has had no uveitis during the four years since this second procedure.

Slit-lamp examination at time of ACT: the large foreign body could be seen in the cataractous lens. One plus flare with no cells and no KP were noted in the anterior chamber. Intraocular tension was 20 by applanation.

Serology: VDRL nonreactive. Darkfield aqueous examination: no spirochetes seen. Aqueous cytology: no cells seen by Leishman's stain of the aqueous. Complete blood count: hemoglobin 15.4, WBC 9,450, neutrophils 65, eosinophils 1, lymphocytes 30, monocytes 4.

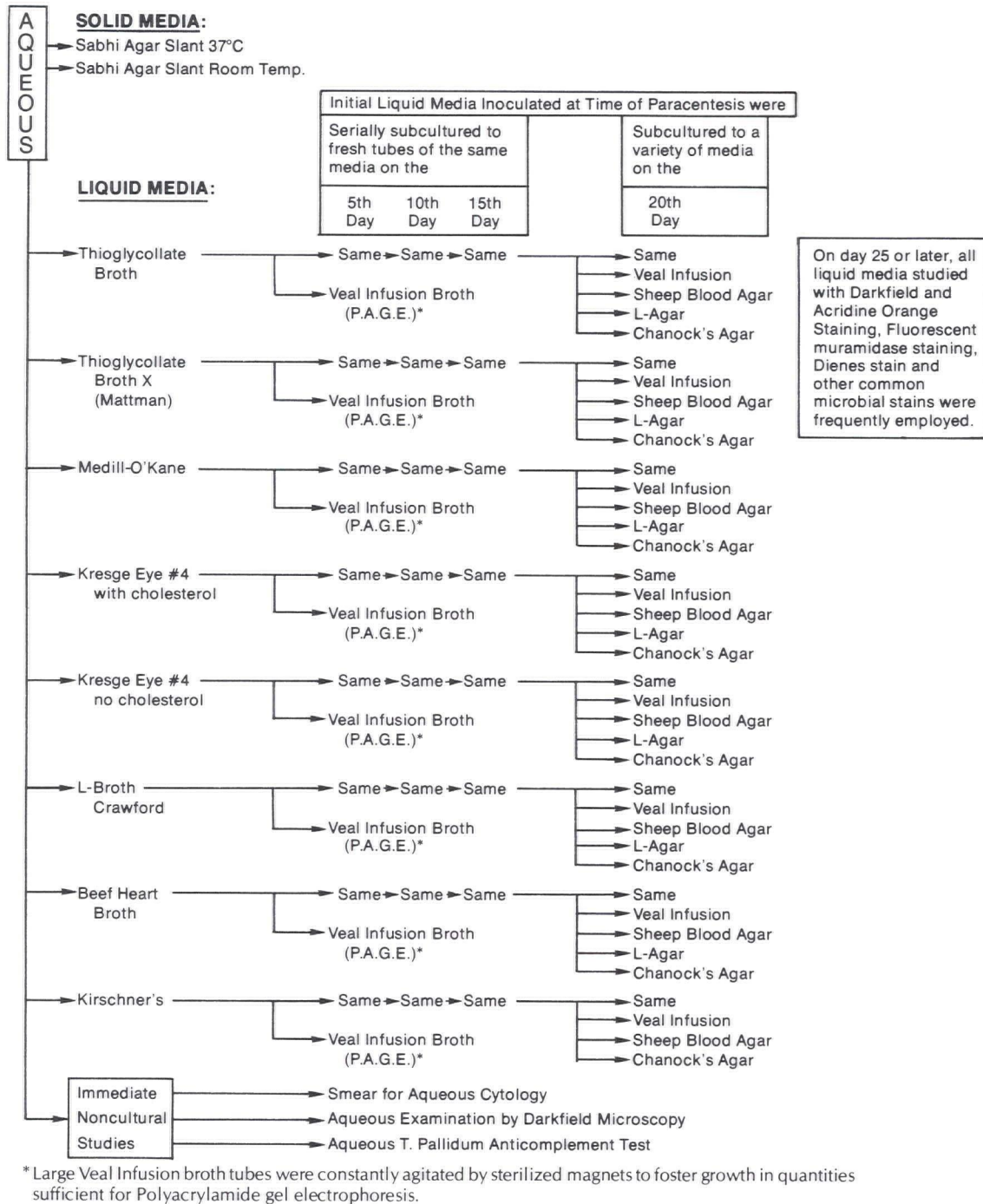


Fig. 2

The complex microbiological subculturing methods used in the third phase of the study are diagrammed on this flowsheet. Variations between this phase and methods used in phases 1, 2, and 4 are described in the materials and methods section.

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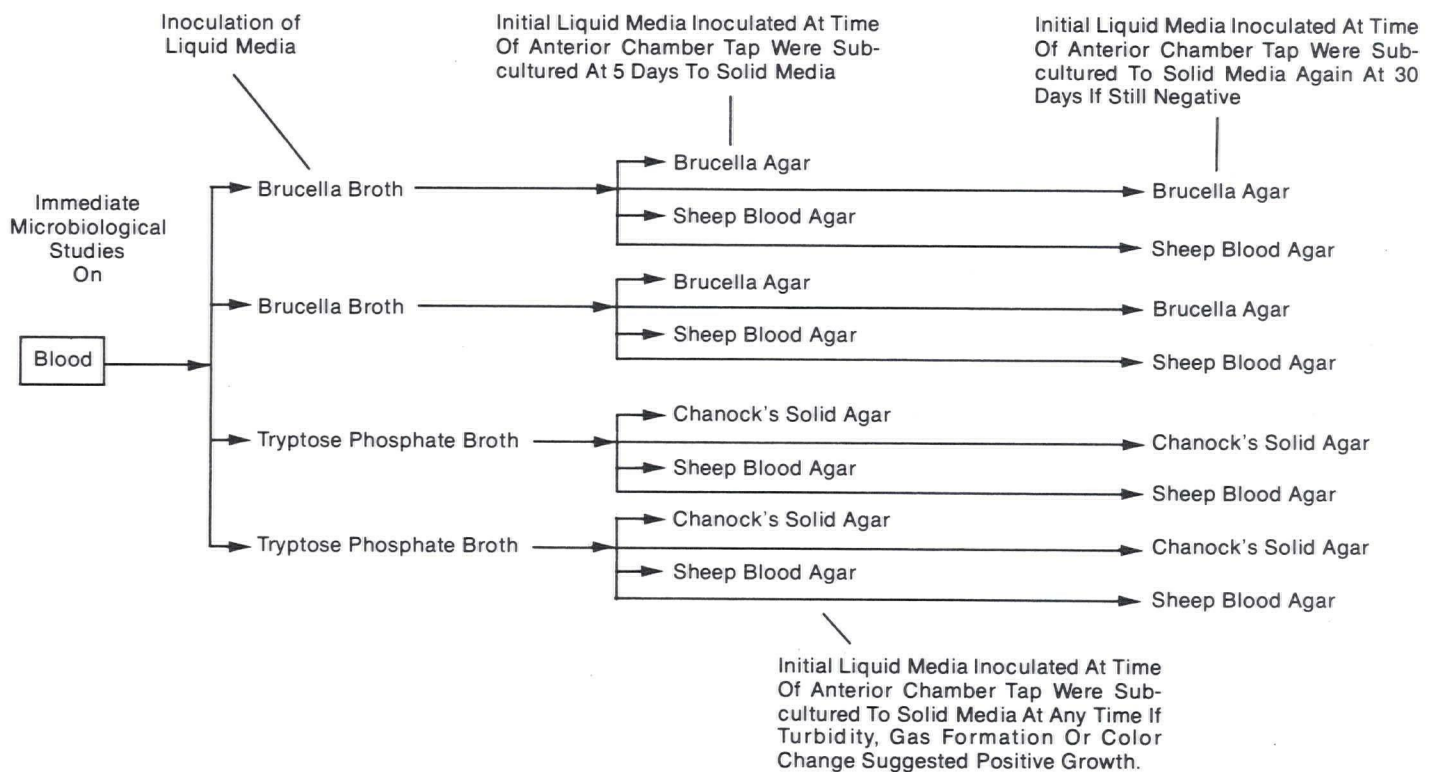


Fig. 3

Microbiological Studies performed on the blood of the fifty most recent patients are diagramed on this flowsheet.

Microbiology

During the first microbiological phase this specimen was inoculated into liquid Medill-O'Kane and thioglycollate media. These were subcultured to various solid agar plates after five and after thirty days of incubation. Chanock's agar plates, subcultured from the initial thioglycollate tube at both five and thirty days, eventually grew out colonies of bacterial CWDF which were acridine orange-positive and which were recorded photographically. The five-day subculture grew out after an additional 39 days, and the 30-day subculture after an additional 24 days. Classical colonies of *Corynebacterium* were recovered from several solid agar plates. From the same aqueous specimen, *Diplococcus pneumoniae* and *Staphylococcus epiderimidis* were also recovered, each on a single medium.

Comment

Exogenous bacteria may enter the eye through injury or through surgical procedure. Although there was no obvious intraocular inflammation in this patient before anterior chamber tap, the eye had been perforated seven days previously by a nonsterile foreign body. In addition to the *Corynebacterium* which grew out on several different media, *Diplococcus pneumoniae* and *Staphylococcus epi-*

dermidis were recovered on one isolation medium. This case, then, represents a polymicrobial infection. In several other patients, several bacterial species and/or more than one bacterial form were isolated. Such polymicrobial populations are probably not uncommon *in vivo* or in the organ under stress. Many species of flora and fauna characterize virtually every ecological milieu.

Hypothesis 4

Alterations in various host factors may subject some persons to a greater incidence of inflammatory episodes including uveitis.

Case 4

Aqueous samples were obtained by ACT from both eyes of a 38-year-old white woman with active anterior uveitis. Her current attack was considered chronic, since each eye had been subjected to previous attacks too numerous to count. At one time a diagnosis of Vogt-Koyanagi-Harada syndrome was considered.

This patient was an obese hypertensive woman whose troubles seemed to begin with an attack of thrombophlebitis following childbirth. After delivery, her gynecologist had great difficulty in clearing a chronic cervicitis.

Three years prior to the ACT she developed streptococcal

pyelonephritis and the following year had two episodes of acute arthritis. Oral surgeons treated her for multiple caries and for chronic periodontitis, eventually removing the infected teeth. General surgeons also treated chronic cholecystitis.

Because of the variety of her infections, skin testing was performed two years prior to the ACT. Three plus reactions were noted to *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus hemolyticus*.

Continuing anterior and posterior uveitis resulted in a blind, painful, phthisical right eye. Three years after the first taps, a second ACT was performed on the right eye just before its evisceration.

Since these microbiological studies were performed, this patient has continued to have infections in several different body systems. She has not had a recurrence of uveitis in her remaining left eye.

Slit-lamp examination at time of ACT: right eye, 1+ cells and flare without KP; left eye, 1+ cells, 2+ flare, no KP.

Serology: VDRL and FTA absorption were nonreactive on multiple occasions. On one occasion the Kolmer and RPCF tests were weakly reactive. Darkfield aqueous examination: no spirochetes seen on any of the three ACTs. Aqueous cytology: Leishman's stain of the aqueous smear from the right eye revealed one lymphocyte per oil field and an occasional mononuclear cell. On the same day, Leishman's stain of the aqueous from the left eye revealed one lymphocyte per oil field and an occasional mononuclear cell. Antinuclear factor: negative. Slit-lamp examination at time of second ACT: 2+ cells, 2+ flare, and no KP. Darkfield aqueous examination: no spirochetes, some small mononuclear cells with rare large mononuclear cells. Aqueous cytology: about 20 large mononuclear cells and a rare lymphocyte per high-power field noted by Leishman's stain of a smear of the aqueous.

Microbiology

Aqueous from the right eye obtained during the first phase of this study revealed classical *Staphylococcus epidermidis*. The bacteria grew out after 28 days on a blood agar plate subcultured from the initial Medill-O'Kane broth. Aqueous from the left eye also yielded *Staphylococcus epidermidis*, in this second instance from the Chanock's subculture plate. No CWDF were recovered from the aqueous in either of these initial specimens.

Aqueous obtained from the second anterior chamber tap of the right eye was studied with phase four microbiological techniques. Bacterial CWDF were recovered after 52 days of incubation from both the L broth and Kirschner's media. These CWDF would not revert. A blood specimen, removed simultaneously with the later tap, grew out transitional form "cocci" from the Tryptose phosphate broth. These were positive by acridine-orange stain, but they also would not revert.

Comments

The prolonged incubation period required before recovery of a classical bacteria after the first ACT may indicate that this isolate was a revertant, perhaps of the same organism which was later recovered as a CWDF which would not revert.

Although this patient's problems with infections have continued, several microbiological studies done by the general hospital microbiology department have reported "no growth".

It seems reasonable to suspect that this patient has some alteration of her defense mechanisms.

Case 5

An aqueous specimen was obtained by ACT of the left eye of a 53-year-old man whose attack of active anterior uveitis had been in progress for one and one-half days. He had had six earlier attacks of iritis, five of them in the previous eight months.

He had had no difficulties with infections until he joined the service in World War II. He was stationed in the Pacific theatre, where he contracted filariasis and scrub typhus. In 1952, he had the first of several episodes of acute pharyngitis. In 1955, a diagnosis of acromioclavicular arthritis was made. On several occasions during this time routine microbiological studies of material from skin lesions were reported as showing "no growth", although this chronic skin problem was diagnosed as "pyogenic granuloma". In 1970, a diagnosis of hypertension secondary to nephrosclerosis was made. In 1971, his first attack of epididymitis occurred with several further episodes thereafter.

In addition to these problems, the patient had had so many "colds" and sinus infections for several years before the ACT that he had resorted to vitamin pills and health foods but without much success.

His eye difficulty, which began several years before the ACT, was first diagnosed as allergic conjunctivitis. Steroid drops were used for several weeks. A series of episodes of left anterior uveitis began eight months prior to the ACT. Each attack responded to cycloplegics and antibiotic/steroid combination drops.

Ophthalmological examination at time of ACT: 2+ cells, 2+ flare, no KP. Serology: VDRL, Kolmer, and FTA absorption tests non-reactive. Darkfield aqueous examination: negative for treponemes. Aqueous cytology: no cells noted on the Leishman's-stained aqueous smear. Antinuclear factor: negative.

Microbiology

Both aqueous and blood, studied by fourth phase microbiological techniques, revealed bacterial CWDF. Tryptose phosphate broth supported the organism found in the blood, while Medill-O'Kane, Mattman's thioglycollate "X" media, and Veal Heart Infusion broth supported the isolate from the aqueous. Both the aqueous and blood isolates were slow to

"come up" and were not recorded as positive until 45 to 60 days following inoculation.

Polyacrylamide gel electrophoresis bands of the organism, obtained after culturing it anaerobically in Veal Heart Infusion broth, were nonspecific.

Comment

This case illustrates anterior uveitis in the presence of multiple systemic infections. Earlier authors would probably have suggested the surgical excision of many of the patient's organs in an effort to extirpate the focus of infection. A variety of alterations of host factors may be responsible for the patient's inability to handle these infections.

Hypothesis 5

Antibiotics used in a regimen similar to that advocated to reduce recurrences of chronic Staphylococcal or Streptococcal disease may ameliorate the pattern of uveitis in some patients, especially if such regimens take into account the cell wall defective forms of these organisms.

Case 6

Aqueous was obtained by ACT from the left eye of a 38-year-old white man with active anterior uveitis. He had had 15 previous attacks. The current attack had been in progress for one day at the time of ACT. His difficulties with anterior uveitis began at age 22, and he thought they were associated with upper respiratory infections. Many episodes of tonsilitis had been treated with antibiotics. Eight years before this attack, he had been hospitalized by another ophthalmologist for evaluation of uveitis, but diagnostic studies at that time were nonproductive. The patient was referred for diagnostic evaluation. An ACT was performed, and local and systemic steroid therapy begun. Within four days, his eye was comfortable, and over the next month the use of steroids was reduced. Three months following ACT, the patient's anterior uveitis recurred in the left eye with cells, flare, and marked injection of the globe, as well as pain and photophobia. This attack was also controlled with steroids.

Two weeks following this attack, an antibacterial CWDF regimen was begun. Erythromycin 250 mg TID and phenoxymethyl penicillin 250 mg QID were each given during alternate weeks.

Fifteen months later, while still on systemic antibiotics, the patient had an upper respiratory infection which was followed by a mild recurrence of the anterior uveitis in the right eye. Antibiotics were continued for another seven months, when he again had an acute upper respiratory infection, followed by a mild recurrence of ocular inflammation consisting of 1+ cells and 1+ flare. Neither of these mild recurrences required treatment; they probably would have been missed had the patient not been followed so carefully.

Twenty-one months after the ACT, he again had an upper respiratory infection, which resulted in a persistent productive cough. Thirteen days later, he had a recurrence of right anterior uveitis with cells, flare, photophobia, and pain. Shortly thereafter, sys-

temic antibiotics were discontinued. The patient has not had a full-blown recurrence of anterior uveitis in the two years since then.

Slit-lamp examination at time of ACT: 2+ cells and 2+ flare with fine KP. Serology: VDRL, RPCF, Kolmer, and FTA absorption tests nonreactive. Darkfield aqueous examination: no spirochetes seen. Aqueous cytology: Leishman's stain of the aqueous smear revealed moderate numbers of both mononuclear cells and basket cells. Antinuclear factor: strongly positive, homogeneous reaction.

Microbiology

Aqueous was inoculated into a microwell plate during the fourth phase of our study. A bacterial CWDF was recovered using thioglycollate, Mattman's thioglycollate "X", Kirschner's, and KEI #4 without cholesterol media. One of these, Kirschner's, remained negative for 84 days. No classical bacteria were recovered from the aqueous either on initial media or by reversion. All studies of the blood specimen remained negative.

Comment

In this case, it seemed that a regimen of antibacterial CWDF antibiotics was effective in interrupting a series of anterior uveitis attacks. A similar effect has been noted in other patients, but in still others antibiotics had no effect whatsoever. In fact, as noted once in this study, some bacterial CWDF are antibiotic-dependent.⁸⁶ During our study of antibiotic sensitivity by the pour-plate technique, an organism was recovered from an aqueous specimen which grew out in numerous, large colonies in media containing erythromycin (two to five units per ml), but not in media containing other antibiotics, or in uninoculated media containing erythromycin.

Case 7

A specimen of aqueous was obtained by ACT of the right eye of a 22-year-old white woman with active anterior uveitis. Her first attack had occurred at the age of 19 following dental root-canal surgery. The attack had been in progress for three days at the time of ACT. During the three preceding years, she had had over 12 attacks of uveitis despite almost constant therapy with local antibiotic-steroid combinations. Her current attack was the fifth on the right eye. During this same period her ophthalmologist had seen her for meibomianitis of the left upper lid and later for a chalazion of the left lower lid. At the time of referral she was under therapy for a vaginal infection.

When the results of the special microbiological studies were obtained, an antibacterial CWDF regimen was started, consisting of alternate weeks of 250 mg penicillin BID and 250 mg erythromycin TID. In the next two years, while on these antibiotics, she had no attacks of anterior uveitis. All steroids were stopped when systemic antibiotics were started. Several days after she discontinued the antibiotics (for financial reasons), there was an exacerbation of anterior uveitis of the right eye with cells, flare, KP,

and pain. She refused an ACT, but resumed taking antibiotics. During one additional year on antibiotics, uveitis did not recur.

Slit-lamp examination at time of ACT: 4+ cells, 4+ flare, and many fine KP noted. Serology: VDRL, Kolmer, FTA absorption tests all nonreactive. Darkfield aqueous examination: no treponemes seen. Aqueous cytology: two neutrophils per oil field and five mononuclear cells per oil field were described on the Leishman's stain of the aqueous smear. There was bridging among the neutrophils and mononuclear cells, and basket cells were described. Complete blood count: hemoglobin 12.9, WBC 10,400. No differential WBC was done within 48 hours of the ACT. Antinuclear factor: negative.

Microbiology

On Mattman's thioglycollate "X", KEI #4 without cholesterol and Medill-O'Kane media, a bacterial CWDF was isolated from this aqueous specimen using the phase four microwell serial subculture techniques. The CWDF eventually grew well in large tubes of Veal Infusion broth agitated anaerobically by magnets for 14 days. Polyacrylamide gel electrophoresis studies of this organism performed at the National Institute of Health by Dr. T. Theodore produced bands which he described as "like a *Streptococcus*". After growth for some time on Veal Infusion broth, the organism was subcultured to blood agar plates and *Streptococcus fecalis* was recovered after 12 days. On Chanock's plates, the organism remained a bacterial CWDF but displayed liquefaction characteristics similar to a *Streptococcus*.

From a Brucella broth tube inoculated with the patient's blood, we recovered a bacterial CWDF whose colonies fluoresced intensely with acridine orange. Although its appearance was described as "coccioid", it would not revert.

Comment

The antibiotic regimen used on some of the patients described in this paper is based on work by Kagan.³³ Erythromycin has its primary effect upon the protoplasmic body of the bacterial cells. This effect, however, cannot be achieved unless the antibiotics penetrate the cell wall. For this purpose, an agent such as penicillin, which exerts its primary influence on the cell wall, is used during alternating periods. Neither agent is as effective when the variant bacterial form is an intracellular guest. Kagan suggested continuing this regimen in chronic Staphylococcal infections for six weeks to three months. An alternative regimen could substitute cephalexin for the penicillin and tetracycline for the erythromycin (Figure 9).

It is probably impossible to clean up any tissue with antibiotics. Godzeski (personal communication) believes that although colistin is the best antibiotic against CWDF in tissue cultures, it will not sterilize them completely of these

forms. Because of our customary drug choices, this opinion is of special interest in ophthalmology for these reasons: (1) it is known that all steroids and immunosuppressants cause classical bacteria to go into the CWDF phase; (2) all alkaloids will increase the rate of induction of CWDF and will also act as stabilizers; and (3) we now know that antibiotics alone will not sterilize any tissues.

Hypothesis 6

The eye in some uveitic situations may be as resistant to microbial eradication by chemotherapeutic or biologic mechanisms as is the kidney in glomerulonephritis or the bone in osteomyelitis.

Case 8

A specimen of aqueous was obtained by ACT on the left eye of a 51-year-old white woman at the time of cataract extraction. She was referred after her first attack of uveitis had persisted for over five months despite regimens of antibiotics, cycloplegics, and local and systemic steroids. Initial diagnoses were bilateral chronic iridocyclitis, bilateral cataract, and hypotony.

ACT preceded an uncomplicated intracapsular cataract extraction. Ocular inflammation was severely increased in the immediate postoperative period. Local steroids and several months of therapy with systemic cortisone were required to control this "severe cyclitis". Persistent postoperative hypotony was accompanied by a serous detachment of the macula. Three months postoperatively, local steroids were finally discontinued. Five months later, the patient still had a nonrhegamentous detachment of the retina with hypotony and papilledema.

Two and one-half years after the first cataract extraction, an uncomplicated intracapsular cataract extraction was performed on the right eye. Cyclitis and vitreitis were reactivated in the operated eye, requiring intensive steroid therapy. The eye developed a pupillary membrane. Final best corrected vision was 20/70 in each eye.

Each time she was admitted to the hospital for these cataract operations, the patient was treated with systemic erythromycin and trisulfapyrimidines for five days.

Nine months after the second cataract operation, she was readmitted with hepatitis. Needle biopsy of the liver provided no definitive diagnosis.

Intraocular pressure by applanation sixteen months after the second cataract extraction was right eye, 8, left eye, 5. Edema of both maculae continued, and inflammation was still being suppressed with local steroid drops.

Slit-lamp examination at time of first ACT: both pupils were bound down to cataractous lenses. Fundus examination with scleral depression revealed marked scarring of the *pars plana* and ciliary body. Intraocular pressure by applanation was right eye, 4 mm, left eye, 3 mm. The left eye had 2+ cells and 3+ flare with fine pigmented KP when examined by slit lamp. The right eye had 1+ cell and 1+ flare.

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Serology: VDRL, Kolmer, and FTA absorption tests nonreactive. Darkfield aqueous examination: no treponemes noted, although many WBCs were seen. Aqueous cytology: many basket cells seen by Leishman's stain of an aqueous smear, along with some small lymphocytes and some large mononuclear cells. Complete blood count: hemoglobin 12.3, WBC 5,700. Antinuclear factor: weakly positive, homogeneous reaction.

Microbiology

Fourth phase studies of aqueous and blood revealed bacterial CWDF in both. Recovery from the blood occurred on only one medium. The organism recovered from the aqueous could be cultured on thioglycollate, Kresge's Eye Institute medium #4 with cholesterol, and Veal Heart Infusion broth. Serial subcultures were made of each of these media from microwells of the same media inoculated with aqueous (Figure 2). The organism did not grow out classical bacteria on blood agar plates but had a coccoid gram-negative appearance on photos made of both the blood isolate and the aqueous isolate. In some media, positive growth was not suspected for 57 days.* The organism could be produced in quantity by constant magnetic agitation of large tubes of liquid Veal Heart Infusion media at 37°C anaerobically. Although the isolated organism was then studied both at the National Institute of Health by Dr. T. Theodore and at this laboratory using polyacrylamide gel electrophoresis techniques, the electrophoretic bands were nonspecific.

Comment

Perhaps a situation analogous to chronic osteomyelitis or pyelonephritis can occur in cases of chronic uveitis which are seemingly uncontrollable by any means. Such an analogy is suggested by the case of this patient from whom bacterial CWDF were recovered from both aqueous and blood. "Gram-positive cocci" were also recovered from the blood on one medium. The nutritional requirements of this transitional form were unknown, however, and it could not be held in culture long enough for speciation.

Hypothesis 7

Sarcoid and its attendant uveitis may be related to a variant *Mycobacterium* species.

Case 9

This specimen of aqueous was obtained by ACT of the left eye of a 32-year-old black man whose first attack of uveitis had been active for over ten months. Six months before the attack, a tissue diagnosis of sarcoid was made following a lung operation. When he was first

* It is important when holding plates or tubes for long periods that they be sealed against drying with masking tape and opened for study only when microscopic examination of the plates reveals colonies which appear suspicious.

seen by an ophthalmologist, his right eye had vision of 20/30 with an occasional large KP but with no cells or flare. The left eye had vision of 20/100 with many large KP, 2+ cells, 2+ flare, many posterior synechiae, cataract, and an intraocular tension of 32. A diagnosis of granulomatous uveitis secondary to sarcoid was made, and therapy with local steroids and cycloplegics was begun. Two weeks later the patient developed severe pain, iris bombé, and intraocular tension of 48. Treatment was unsuccessful, and a peripheral iridectomy was performed. The glaucoma was temporarily controlled, but the inflammation remained. One month later, the pressure was again markedly elevated. One plus cells and flare were noted in the anterior chamber by slit-lamp examination, and many synechia were seen in the angle by gonioscopy. A cyclodialysis again temporarily controlled the glaucoma, but the inflammation continued. Although steroids were used intensively, 3+ cells and flare were usually seen. A second ACT was performed on the left eye four months after the onset of inflammation. One month after this ACT, the cyclodialysis of the left eye closed off, as the inflammation continued.

Six months after inflammation of the left eye had begun, an unplanned extracapsular cataract extraction was performed. Following this operation, inflammation continued for three months despite the use of retrobulbar, local, and systemic steroids. As a result, erythromycin and tri-sulfapyrimidines were used during alternate weeks, continued for six weeks, and then discontinued. The eye quieted down and remained so for ten months after the antibiotics had been discontinued. Then anterior uveitis recurred, and a second ACT was performed on the left eye.

Slit-lamp examination at time of ACT: 3+ cells, 3+ flare, many fine and some large KP. Serology: VDRL nonreactive. Darkfield aqueous examination: negative for spirochetes. Aqueous cytology: two mononuclear cells and an occasional lymphocyte per high power field noted on the Leishman stain of the aqueous smear. Complete blood count: hemoglobin 13.7, WBC 8,700, neutrophils 61, lymphocytes 33, monocytes 3, eosinophils 1, basophils 1, bands cells 1. Ophthalmological examination at time of second ACT: 1+ cells, 1+ flare with no KP. Serology: all tests nonreactive. Darkfield aqueous examination: negative for treponemes. Aqueous cytology: Leishman's stain of the aqueous revealed a rare eosinophil and an occasional mononuclear cell. Antinuclear factor: medium homogeneous reaction.

Microbiology

These microbiological studies were performed during phase one. Bacterial CWDF were recovered from both of the initial liquid media (Medill-O'Kane medium and thioglycollate medium) inoculated at the time of the first ACT. Subcultures at five days to plates of Chanock's solid agar all showed growth after an additional 11 days. Suspicious areas of agar stained positively with acridine orange (Figure 8B) and Dienes stain, and were photographed. This organism would not revert. It could be grown in quantity sufficient for polyacrylamide gel electrophoresis, which showed nonspecific electrophoretic bands. No organisms were recovered from the second aqueous specimen.

Comment

This patient, with sarcoid established by biopsy, also had bacterial CWDF recovered by ACT. Systemic antibiotics seemed to halt the inflammation.

Dr. L. Mattman studied this isolate and found it to be acid-fast (Figures 8C, F). She felt it was a Mycobacterial CWDF and that it was either "identical or very similar to organisms we are studying from three other cases of sarcoid." The cessation of this patient's uveitis was of interest since some Mycobacterial species are known to be sensitive to erythromycin. (In the British Isles, erythromycin is considered therapeutic for children infected with *Mycobacterium avium*.)

Dr. L. Mattman found that in mice prestressed with cortisone this organism was lethal when injected intraperitoneally. She could later recover it as a CWDF from various abdominal sites, but could not recover it as a classical pathogen. Control mice, prestressed with twice as much cortisone and injected intraperitoneally with the media, did not die.

Case 10

An aqueous specimen was obtained from the right eye of a 45-year-old white man with active generalized uveitis. The attack had been in progress for one month. Nine months before this attack, he had posterior exudative chorioretinitis in the right eye. Chest x-rays revealed "old and inactive TB". The patient had been on systemic steroids for many months for sarcoid, a diagnosis established by biopsy.

Examination of the right eye at time of ACT: slit lamp showed 1+ cells, 1+ flare, and many mutton fat KP. Serology: VDRL, FTA absorption tests nonreactive. Darkfield aqueous examination: negative for treponemes, although small mononuclear cells as well as large mononuclear cells trailing cytoplasm were noted. Aqueous cytology: intact and disintegrating mononuclear white blood cells were seen and seemed to be chaining or bridging in alternating rather than random fashion. Antinuclear factor: weakly homogeneous reaction.

Microbiology

Bacterial CWDF were recovered from cultures of both aqueous and blood performed during the fourth phase of this study.

Within 39 days of the initial aqueous inoculation, acridine orange studies of the thioglycollate and of the Kresge Eye Institute #4 with cholesterol media were positive and were photographed. An organism from this same aqueous specimen, recovered from Kirschner's TB media (Figures 8D, E), fluoresced with the Truant Auramine-Rhodamine TB stain (Figure 8A), but could not be grown as a classical bacteria. The CWDF recovered from the blood were grown out on Tryptose phosphate broth.

Comment

This study seems to corroborate the relationship which has already been suggested between sarcoid and bacterial CWDF. The CWDF may be mycobacterial.

Discussion

Cell wall defective forms

The study of bacterial cell wall defective forms presents substantial problems. Clinical isolates are delicate, fastidious, and easy to "lose". The start-up period for a new isolate, especially from a clinical source, is often slow. Growth, fairly well established one day, weakens and dies the next. It is unwise to discard cultures in a week or so, as happens with plates and tubes in a general microbiological laboratory. Clasener⁸⁶ noted:

The awareness of a need for special nursing of such microorganisms has led to an extension of diagnostic possibilities of clinical bacteriology. This important step does not imply a breakthrough in techniques, however. Extension of routine bacteriological examination by the use of media supplemented with serum and osmotically protective substances is only a minor step. Longer incubation and microscopic examination will mean a considerable increase in the work load. The rich medium and long incubation make high demands upon sterile techniques. Most difficult, however, will be the interpretations of the findings. Personal experience is extremely important. Continuity of personnel and facilities, therefore, will be a key word in this field. Transition from research to routine procedure will be very difficult.

The newer microbiological techniques demand tremendous time and effort. A wide variety of media are required. Both liquid and solid media must be examined microscopically for several months, and many uninoculated control plates, tubes, and media batches are necessary. Plates negative for classical bacteria should not be discarded after five days since bacterial CWDF are usually not seen so quickly. In aseptic meningitis, for example, cultures negative at five days might be positive for CWDF if held for several weeks.³¹ Most laboratories hesitate to incubate negative plates for long periods, although workers will readily hold slants for six weeks when the acid-fast bacillus is suspected.

Although this seven-year study began with a fairly simple and unsophisticated approach, during later, more complex phases, seven or more media, often involving 50 to 80 tubes, plates, or microwells, were used for each aqueous or blood specimen. (Figure 2). All media chosen were recommended in the literature, except for Kinsey's media,* which were added because of their close similarity to aqueous.

* In a subexperiment, it was found that Kinsey's media will support many classical bacteria and the CWDF of *Streptococcus*, *E. coli*, *Staphylococcus*, as well as *Mycoplasma hominis*.

Studies on Uveitis

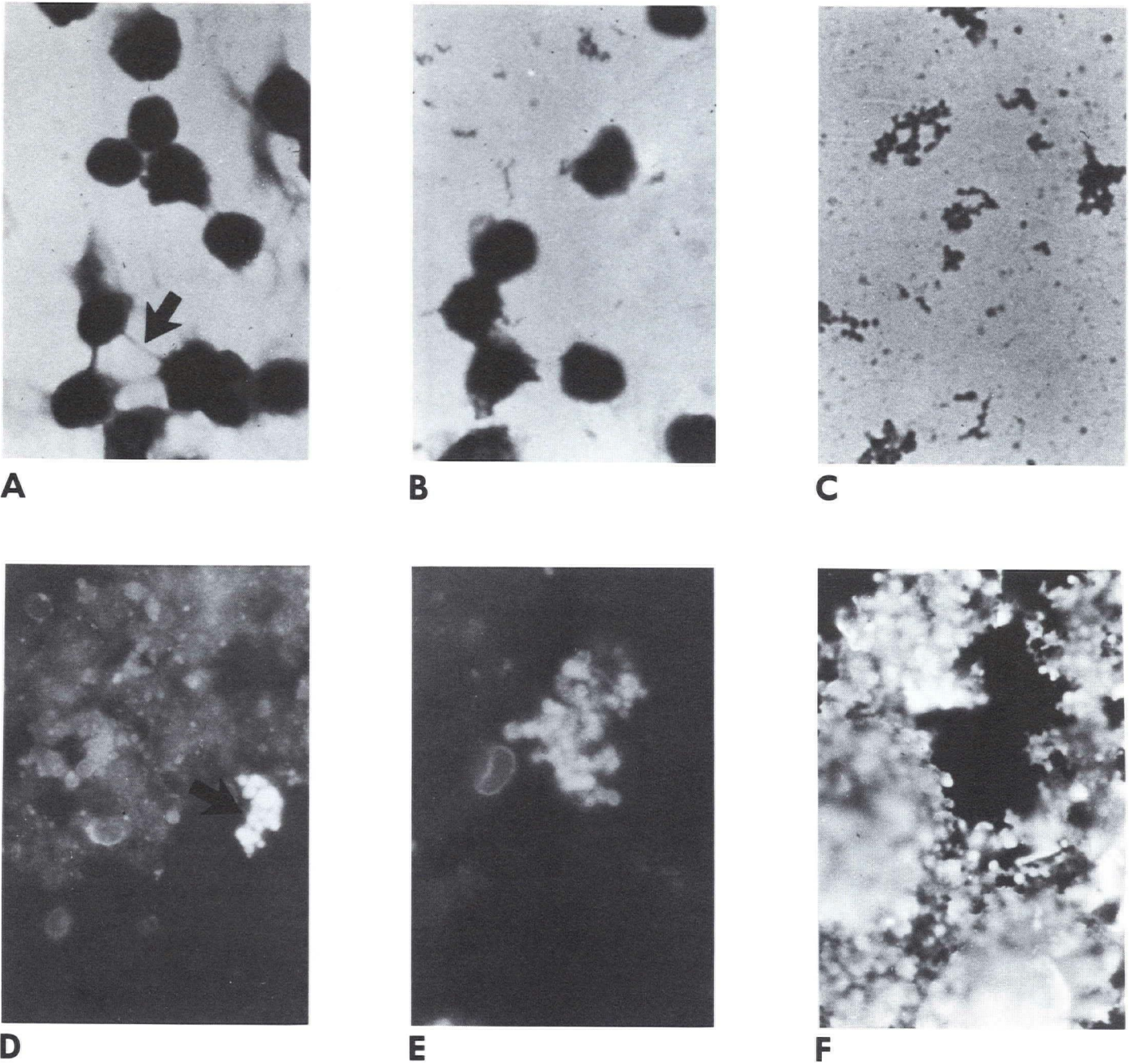


Fig. 4. Case 1

A. Aqueous smear revealed neutrophils with some fibrin strands. Cytoplasmic bridges (arrow) may be seen joining several neutrophils. No organisms seen. (Leishman X1,200). B. Mononuclear and neutrophilic leukocytes with amorphous debris. (Leishman X1,200). C. Another area of same smear 4A and 4B showing transitional bacterial forms in "Cocoid", irregularly-sized, variably staining bacterial forms. (Leishman X 13,200). D. Intensely fluorescing CWDF colony (arrow) growth on tryptose phosphate broth from blood. (Acridine orange X 540). E. Bacterial CWDF from aqueous on thioglycollate broth. (Acridine orange X1,000). F. Intense fluorescence of large bacterial CWDF colony. Isolate from blood on tryptose phosphate broth. (Acridine orange X 1,000).

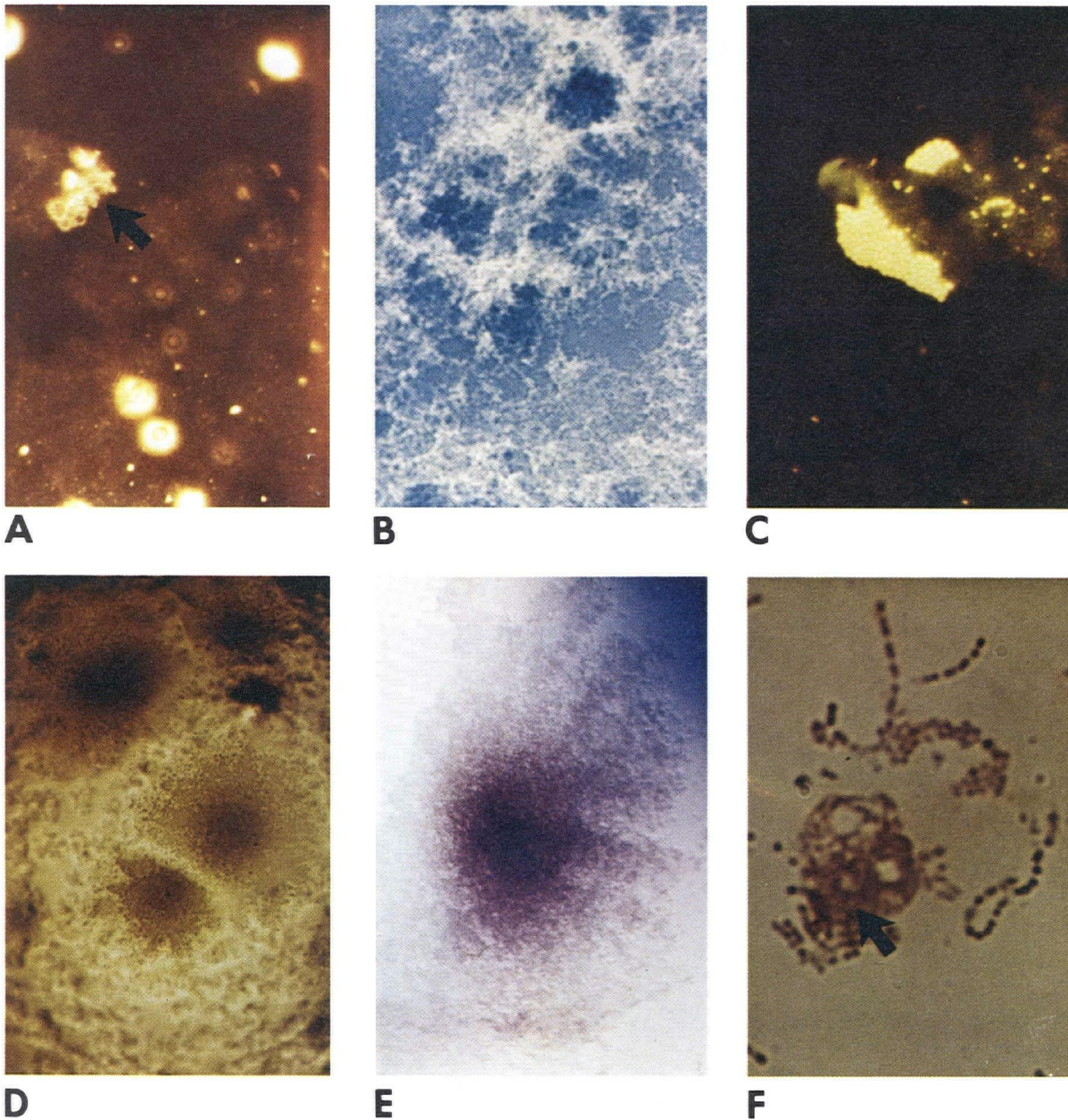


Fig. 5. Case 7

A. Liquid thioglycollate inoculated with aqueous became cloudy with wispy strands. Varied-sized, sticky spheres (arrow) like fish eggs resemble CWDF colony. (Darkfield X 1,200). B. Spheres from (A) appear as dark blue clumps when stained with Dienes (arrow) stain. The medium alone is stained pale blue forming the background. (Dienes X 1,200). C. Fluorescent CWDF colony. Media, devoid of nucleic acid, do not fluoresce. (Acridine orange X 1,200). D. Colonies resemble "fried egg" morphology on Chanock's agar subcultured from aqueous in heart infusion broth. (Unstained X 40). E. CWDF colonies appear dark blue and granular. (Dienes X 100). F. Amorphous, irregular CWDF colony (arrow) attempting to chain as a transitional form "coccus". (Gram X 3,200). Probably a reverting *Streptococcus*. Figures 5D, E, and F are studies from a case of anterior uveitis not presented in Case Reports.

Studies on Uveitis

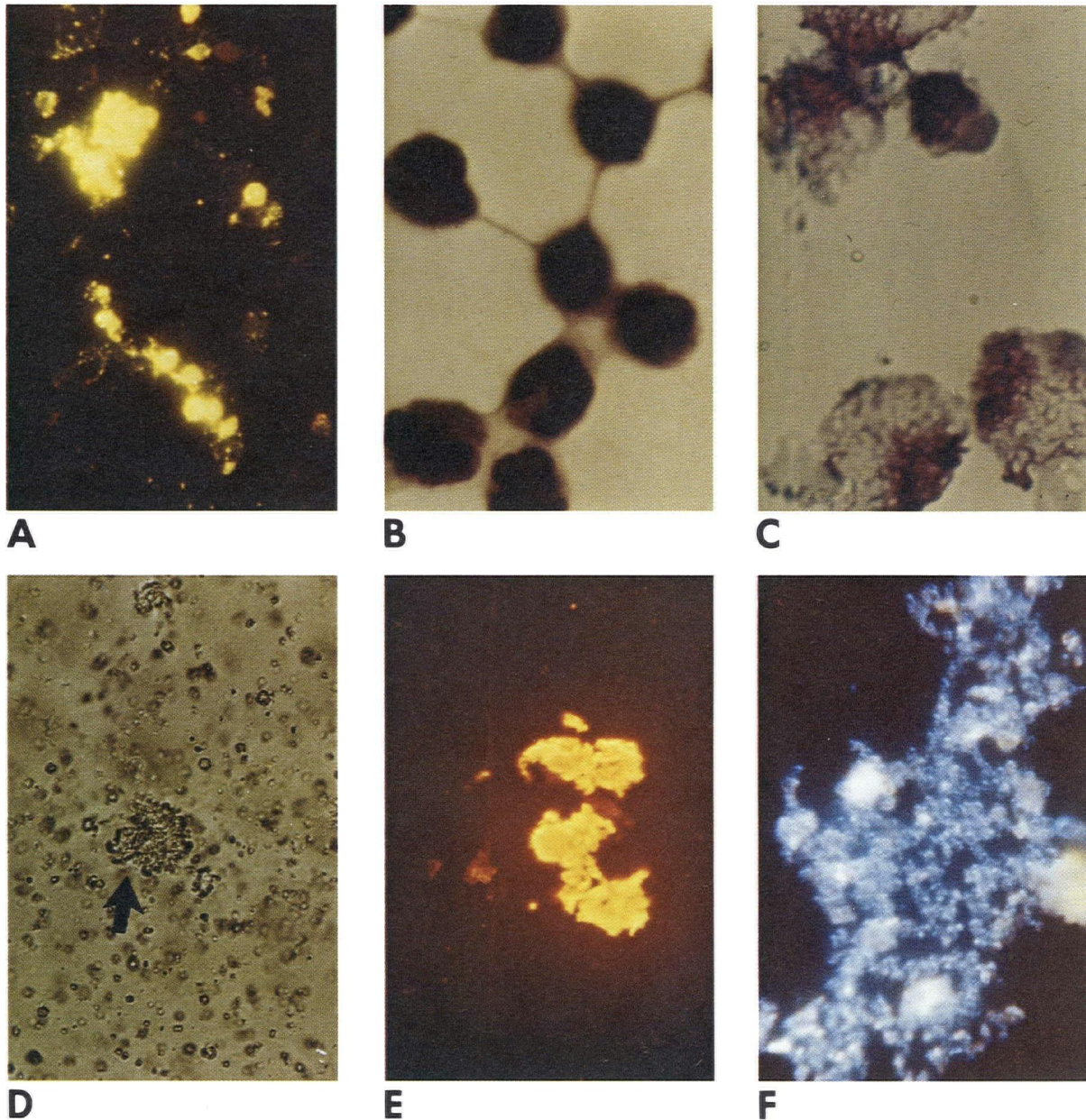


Fig. 6

A. Leukocytes in aqueous. (Darkfield illumination X 1,000). B. Neutrophils and mononuclear leukocytes in smear of aqueous. Note cytoplasmic bridges. (Leishman X 1,600). C. Basket cells or vacuolated neutrophils. Note degenerate, vacuola appearance of spent leukocyte in smear of aqueous. (Leishman X 4,000). D. Bacterial CWDF (arrow) colony on solid Chanock's agar. (No stain X 160). E. Intense fluorescence of CWDF colony from same solid agar plate as Fig. 6D. (Acridine orange X 500). F. Bacterial CWDF colony stains blue in areas completely free of cell wall muramic acid and "gold" where muramic acid exists in cell wall. (Rhodamine muramidase by combined UV and Tungsten light X 500).

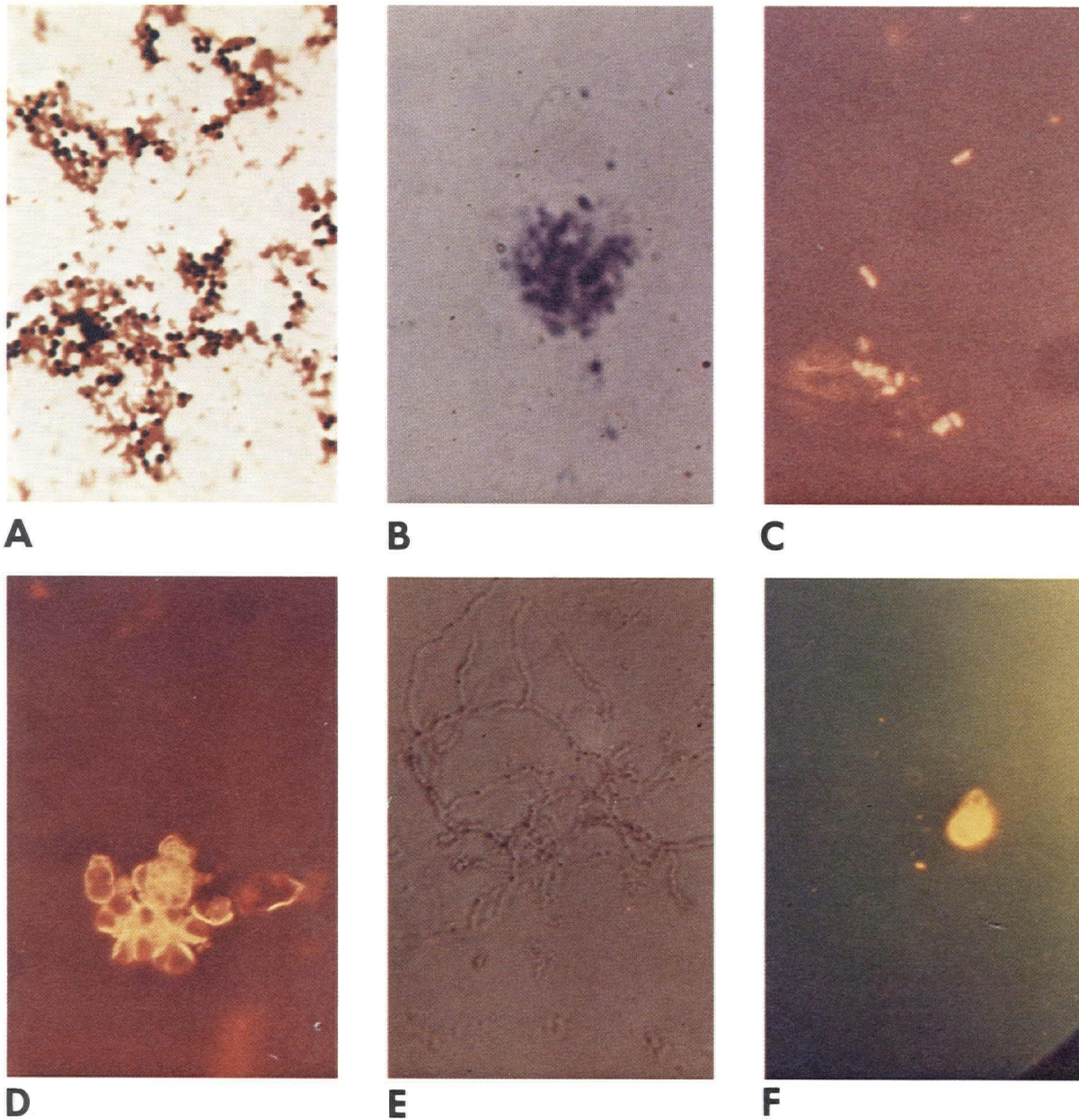


Fig. 7

A. Semireverted or transitional form cocci. Such forms were sometimes followed by complete reversion but often had unknown nutritional requirements and could not be maintained in culture. (Gram X 1,600). B. Bacterial CWDF growth from Chanock's solid agar plate; stain placed directly on agar surface. (Dienes X 1,600). C. Fluorescing organisms which may be "trying to revert to a small bacillus" isolated from aqueous in thioglycollate broth. (Acridine orange X 1,600). D. Budding colony of fluorescing CWDF. Aqueous isolate from patient with Eale's disease and recurrent uveitis. (Acridine orange X 3,200). E. *Nocardia dassonvillei* (Phase contrast X 1,200). F. Fluorescence of budding CWDF colony isolated on Chanock's agar from initial thioglycollate broth inoculated with aqueous. (Case 6). (Acridine orange 540 X).

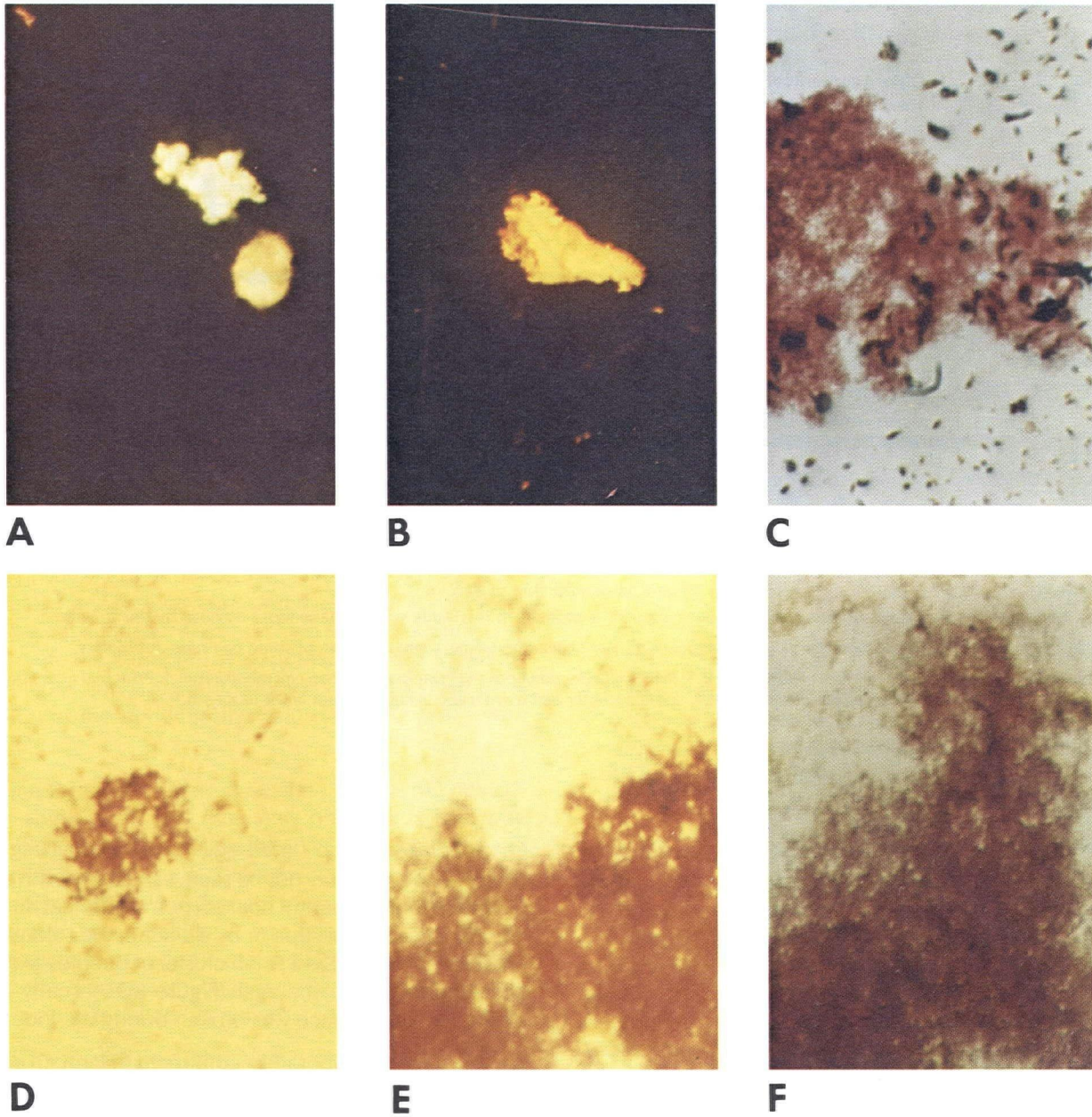


Fig. 8: Sarcoid

A. Grapelike cluster of greenish-yellow staining bacterial CWDF in direct smear of aqueous from patient with active uveitis and known sarcoid. (Case 10). Round body below colony is a leukocyte. (Auramine Rhodamine X 1,600). B. Colony of CWDF from aqueous of patient with sarcoid and active uveitis, Case 9. (Acridine orange X 500). C. Acid-fast stain of CWDF colony from Case 9. (Hauduroy X 440). D. Acid-fast staining of colony of CWDF isolated from aqueous of Case 10. (Hauduroy X 440). E. Acid-fast CWDF colony from aqueous culture of Case 10. (Hauduroy X 440). F. Acid-fast colony from aqueous culture, Case 9. (Hauduroy X 440).

S. AUREUS . . . SCHEMATIC CROSS-SECTION
Primary Sites of Action of Antibiotics

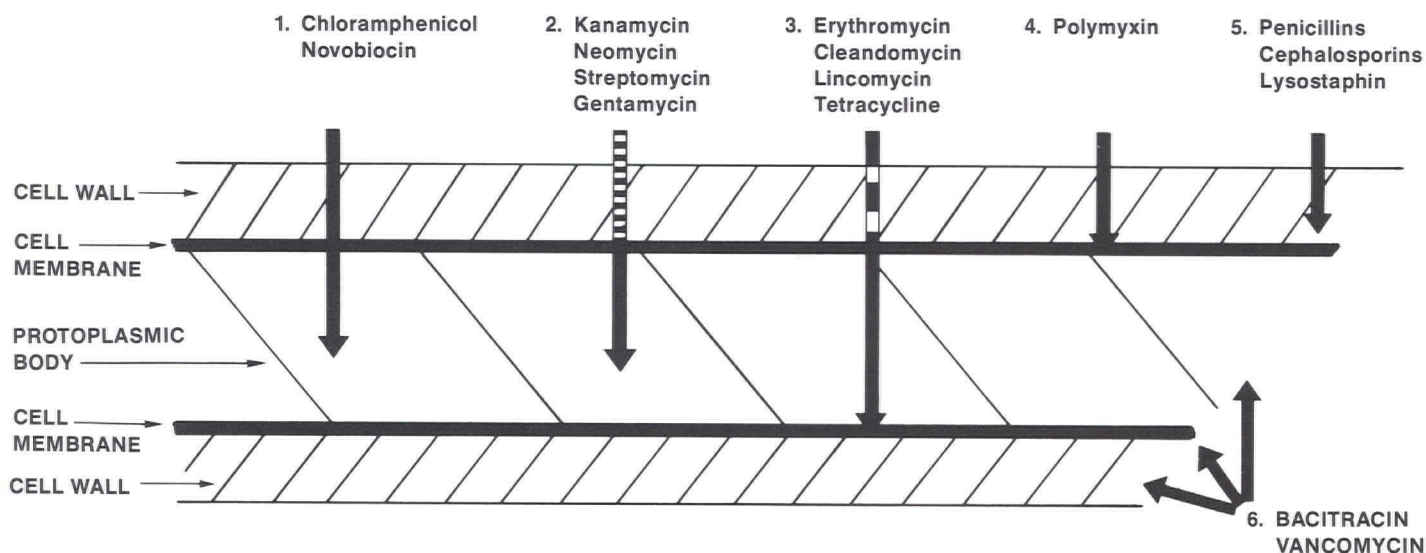


Fig. 9. Antibiotic Site of Action.

Rationale for the use of various antibiotics on some of the patients in this study will be made more obvious by this diagram.

The schematic cross-section of *Staphylococcus aureus* shows the primary site of action of some common antibiotics.

1. Cell wall has little, if any, effect; 2. Cell wall retards effect; 3. Principal effect on body; 4. Principal effect on cell membrane; 5. Principal effect on cell wall; 6. Principal effect on cell wall, some on body and membrane. Adapted from work of Kagan.³³

It would be valuable to ascertain which CWDF media were most effective with small clinical specimens. Unfortunately, all media grew some organisms, although no medium grew every organism. Of the bacterial CWDF isolated, about 70% grew on Medill-O'Kane, Kirschner's, or beef heart infusion broth; about 60% grew on Kinsey's Kresge Eye Institute medium #4 with cholesterol, thioglycollate "X" medium or thioglycollate; about 50% on Crawford's L-broth; and about 40% on Kinsey's Kresge Eye Institute Medium #4 without cholesterol. The use of many media is necessary. Those interested in variant microbiology are now at the stage where those studying Mycobacteria were about two generations ago: although something is known about many media, a single, "all purpose" CWDF medium has yet to be found.

Suspicion of microbial activity exists when a color change occurs in some liquid media, when clouding or wispy material appears in a clear medium, or when gas bubble formation occurs. To be significant, such changes had to be noted *only* in the inoculated tube or microwell, and not in the control tube or microwell. Often such changes will be seen only when the tube is shaken and studied against a bright light or with slit-lamp illumination using the Tyndall effect.

On solid media, the "fried egg" type colony (Figure 5D) usually expected may not be seen, especially with cell wall defective forms recently coaxed from a clinical specimen. Seen under a microscope or by slit lamp, the colony is often granular or appears as a small cluster of bubbles growing on and especially into the agar (Figure 6D), usually at a site where the agar surface was broken during the inoculation.

Early work in this laboratory confirmed that much commercial agar has an inhibitory effect⁸⁸ which varies greatly from lot to lot. For this reason, Chanock's solid agar was abandoned as an initial isolation medium. However, its use for final serial inoculations was continued since CWDF colonies, when they do grow on Chanock's agar, are often so typical morphologically that their identification is facilitated. Difco[®] purified agar was used, as pretesting indicated a relative lack of inhibitors with this brand. In addition to the inhibitory effect of agar, other common artificial and natural media are known to suppress CWDF. In part, this explains why, although aqueous specimens have been cultured previously on many media, the chances of recovering

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fastidious organisms were poor, even if these media had been held for long periods under anerobic conditions.

Acridine orange is a cytological stain which produces fluorescence of nucleic acids. It was first described by Bertalanffy⁸⁷ in 1956, and used in the *in vitro* study of bacterial L-forms by Hui in 1969.⁹⁴ Deoxyribonucleic acid (DNA) stains bright, light green. Ribonucleic acid (RNA) stains orange to fire red. Young colonies (Figure 4F) fluoresce red (RNA); as they age, DNA increases and the fluorescence becomes greener. Acridine orange staining alone⁸⁹ is inadequate for definitive statements and must be coupled with morphology. Prior to beginning clinical isolations, we studied its various pitfalls and artifacts by using it on cultures of known bacterial CWDF.

The absence of a cell wall makes CWDF difficult to describe morphologically, yet morphology is essential in the interpretation of acridine orange staining. Fluorescent staining without morphology is meaningless; morphology without fluorescence equally so. The presence of three or more fluorescing colonies with acceptable morphology was necessary before a slide was considered positive for CWDF. Morphological characteristics to which some credence was attached were the clumping of grapelike or ovoid bodies of various shapes and sizes into bunches like sticky fish eggs (Figure 4E), or the budding or cystic subdividing of forms (Figure 7D).

Rhodamine B-tagged muramidase stain (Figure 6F) detects muramic acid in cell wall fragments on atypical bacterial variants. In all but two instances the CWDF isolated showed fluorescence with this stain. Since mycoplasma do not take this stain, the CWDF were thought to be bacterial in origin. Where growth was positive, it was generally strongly so, with large numbers of bodies seen in liquid media whose control showed no growth. Where evidence was equivocal, the specimen was called "negative".

Although there was less confidence in organisms which could be isolated on one medium alone, such data were not discarded. Growth on one medium only may mean that a fastidious organism is fostered only by the nutrients in that medium, that inhibitors of growth are present in the other media but not in that one, or that the isolate is a contaminant.

Consistent reversion of cell wall defective bacterial forms to the parent bacteria would simplify identification. Many of these isolates would not revert, although most of the classical bacteria isolated were probably revertants. Such reversion was spontaneous and unrelated to our attempt to obtain reversion. Media are not available to induce reversion consistently to the parent classical bacteria. When bacterial CWDF do revert, it is usually a fortuitous occurrence which

the investigator, though pleased about, is at a loss to explain. CWDF, especially if induced by lysozyme, may be unrevertable or stable.⁹⁰

Calderone⁹¹ believes that reversion can be achieved by gradually reducing the concentration of whatever material converted the classical bacteria to its variant form (penicillin, serum, etc.). Though attractive, the theory has yet to be fully tested. It is notable that Calderone obtained reversion only when the primary growth had been in a penicillin medium. His isolation and reversion techniques do not form a substantive part of this study.

Classical bacteria

These studies confirm a paucity of classical organisms in anterior uveitis even when no such paucity of bacterial variants exists. The variety of classical organisms isolated was wide. If the organisms isolated were those commonly seen on the conjunctiva, they would have been expected to grow out quickly (within a week) on sheep blood agar plates. Plates were held for 30 days or more and colonies studied with routine microbiological tests. Plates or tubes on which classical bacteria grew were transferred to a routine microbiological laboratory for identification and speciation.

The number of isolations of classical bacteria compared favorably with those in European studies.^{18,19,20} Classical bacteria were recovered from the aqueous of those patients with CWDF in the aqueous more frequently than from those patients without CWDF in the aqueous. The following classical bacteria were isolated: *Corynebacterium*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas* (not *aeruginosa*), *Mima polymorpha*, and *Streptococcus fecalis*. Each was recovered from the aqueous of one patient, with the exception of *Staphylococcus epidermidis*, which was isolated in four separate patients. In each instance, these classical bacteria were isolated on more than one medium. Isolations of classical bacteria on one medium alone included *Diplococcus pneumoniae*, a nonhemolytic *Streptococcus*, *Pseudomonas maltophilia*, *Nocardia dassonvillei*, *Escherichia coli*, and *Pseudomonas alcaligenes*.

Transitional bacterial forms

In addition to those classical bacteria which could be fully speciated and probably represent some revertants as well as other classical bacterial isolates, a number of transitional bacterial forms (Figure 7A) were also recovered. Because these forms have incompletely known nutritional and environmental requirements, they could not be held in culture long enough for full speciation. Such transitional forms included gram-positive cocci, gram-positive rods, "classicals not otherwise identified", "cocci", a coliform orga-

nism, and a gram-negative rod (Figure 7C). The last two were isolated on one medium only. The gram-positive cocci were isolated from four different patients in several media.

Although all CWDF stain gram negatively, these transitional forms could represent a wide range of pathogens. Some of the transitional forms were probably variant forms of common pathogens such as *Staphylococcus aureus* or *Beta hemolytic streptococcus*.*

Mycobacteria Tuberculosis

The "tuberculosis" theory⁹² of uveitis has long been dead, and little has been learned in this study that would revive it. Initially, three agar slant media were used in attempts to isolate *Mycobacterium tuberculosis*. They were American Trudeau Society medium, Petrognani medium, and Lowenstein-Jensen medium. In later studies, these were replaced by Kirschner's medium, since there is no literature to substantiate the use of the other three media for the isolation of the Mycobacterial CWDF.

The Truant⁵⁹ stain for *M. tuberculosis* (Auramine-Rhodamine stain) was used on most slants suspected of harboring Mycobacteria. The technique produces fluorescence of TB and of TB CWDF. No organism was isolated that, with either Auramine-Rhodamine or acid-fast stains, resembled a classical Mycobacterium.

There is evidence of an association between sarcoid and Mycobacterial CWDF.^{62,64} In one aqueous specimen, CWDF were isolated from a patient with sarcoid. They were not thought to be classical Mycobacteria since they would not colonize on the surface, but they were suggestive of Mycobacterial CWDF as they accepted Auramine-Rhodamine stain.

Fungi

After the early phases, the more familiar Sabouraud's maltose agar medium was replaced with Sabhi medium since, allegedly,⁶⁰ it will support most stock cultures of pathogenic fungi, including *Histoplasma capsulatum*, *Coccidiomyces immitis*, and *Blastomyces*, when used at room temperature or 37 C. All cell wall defective form media, such as Mattman's "thio X" media, Medill-O'Kane, and Hinton's beef heart infusion broth (HIB), contain various animal extracts or amino acids, and although they are compounded to present

the fastidious bacterial forms with suitable nutritive and hypertonic conditions, they will also support many fungi. Despite the use of media considered ideal and a long incubation period at both 37 C and at room temperature, not a single fungal isolate of interest was obtained.

Spirochetes

Kinsey's media were used in the hope that, if organisms especially of the genus *Spirochaeta* do survive in the eye, perhaps they might be recovered with media closely resembling aqueous. Kinsey's synthetic media were used with and without cholesterol since *Mycoplasma* require cholesterol for growth, while classical bacteria and bacterial CWDF do not. Suggestive evidence was obtained that the Kresge media might support spirochetes. On several occasions, in patients in whom *Spirochaeta* was identified in aqueous by darkfield, "spirochetoid forms" were noted growing in the Kresge Eye Institute medium. No other media ever grew out spirochetoid forms. The morphology of such spirochetoid forms was not completely classical. *Spirochaeta* have a variant CWDF,²⁵ and perhaps the presence of this form in the eye explains some of the enigmas of ocular syphilis.

In a subexperiment with commercially obtained classical *Treponema pallidum* (Reiter's strain), mature, wall-intact *spirochaeta* did not fluoresce. Although the classical *Treponeme* does not stain with rhodamine muramidase, its CWDF may. In three aqueous specimens, all from patients with no spirochetes in the aqueous but with some evidence of syphilis, there were spheroplasts or CWDF which absorbed the muramidase stain. Perhaps these were *T. pallidum* CWDF.

Contaminating organisms

Media used to culture CWDF are so enriched that contamination cannot be completely avoided, especially when cultures are held for long periods. Bacillus species and common saprophytic molds were discarded as contaminants. In no case had smears made directly from the aqueous suggested that such fungi or bacilli were present.

Summary

During four successive phases over a seven-year period, increasingly complex microbiological studies were performed on aqueous and blood specimens taken from patients who had uveitis and from others who had no signs of this ocular inflammation. Anterior chamber tap was done on 134 eyes of 118 patients. The microbiological studies were also correlated with some serological, hematological, and cytological studies. Fifty patients had microbiological studies performed on the blood as well as the aqueous.

* Clinicians should be aware that the initial gram-stain characteristics of a clinical isolate do not have irrevocable validity. It has previously been shown with bacterial ulcers of the cornea that the first gram-stain impression can be highly unreliable.⁹⁹ In ulcers later proven due to *Pseudomonas aeruginosa*, initial gram smear results of gram-positive cocci, gram-negative cocci, gram-positive rods, and gram-negative rods have been reported.

Some nonmicrobiological blood studies were performed on almost all patients. Most had serological tests for syphilis and antinuclear factor tests; many had a complete blood count, and some had an erythrocyte sedimentation rate, and a lupus erythematosus test. Spirochetes were sought in the aqueous. Media used would have supported *Mycoplasma* had they been present.

To analyze these findings, the same patients were regrouped as follows: (1) all patients; (2) patients arranged according to the anatomical variety of uveitis; (3) all patients with active anterior uveitis and active generalized uveitis; and (4) patients subdivided for those with and without aqueous bacterial CWDF. These subgroups were then compared, with some 40 different parameters used.

Attempts were made to correlate the presence of variant bacteria in the aqueous to factors in the history, the ophthalmological examination, the serology, the blood, and the aqueous cytology.

Forty percent of the aqueous specimens grew out cell wall defective bacterial forms, and classical bacteria were recovered from 12%, proving that the aqueous is not universally sterile since it supports these bacteria. Confirmation of bacterial CWDF growth was by fluorescence-staining techniques, by reversion to classical bacteria and, sometimes, by polyacrylamide gel electrophoresis.

A statistically significant difference could not be found between the incidence of CWDF in the various anatomical types of uveitis. However, this does not mean that differences could not occur, since the group without uveitis was composed primarily of persons hospitalized for other medical or surgical eye problems. Hence, they were not a group of healthy controls.

There were hints that the presence of CWDF in the aqueous was more frequent in younger patients with uveitis. CWDF also seemed to be more frequent early in an attack, or if there were foci of infection, a history of arthritis, sarcoid, or diabetes. Neither the number of previous attacks of uveitis nor the sex of the patient seemed related to the presence of CWDF.

Some findings in the ophthalmological examination seemed to forecast the presence of bacterial CWDF in the aqueous. Although positive aqueous microbiological studies were unrelated to the anatomical variety of uveitis, the slit-lamp presence of many aqueous cells or of a dense aqueous flare, or of hypopyon, seemed to be significant. Mutton fat KP ("epitheloid and histiocytic mononuclear phagocytic cells")⁶¹ also may have pointed to their presence. Old, crenated KP indicated that CWDF were no longer present.

Some correlation may have existed between the presence of aqueous CWDF and certain leukocytes in the anterior chamber. Neutrophils (either with other leukocytes or as the predominant aqueous cell), vacuolated or "basket" cells, and macrophages all seemed to suggest the presence of bacterial CWDF. The aggregation, chaining or bridging of leukocytes (which occurs when phagocytosis is particularly efficient) was seen more frequently when bacterial CWDF were not present in the anterior chamber.

Serological tests for syphilis showed the FTA absorption test to be the most sensitive, the VDRL least sensitive. Aqueous spirochetes were only occasionally present despite many other indicators of syphilis. This study did not confirm the high incidence of spirochetes found by others.

Patients with anterior uveitis had increased incidences of anemia and more frequent abnormalities in the shape, size, and hemoglobin content of erythrocytes. Patients with generalized uveitis more often showed abnormally low platelet counts, higher erythrocyte sedimentation rates, and higher neutrophil and lymphocyte counts.

There may be some correlation between the peripheral white blood count and the presence of aqueous CWDF since neutrophilia, leukocytosis, and erythrocyte abnormalities were more frequent when CWDF were found.

Conclusions

1. The aqueous is not always sterile.
2. Specialized media and improved microbiological techniques will isolate cell wall defective bacterial forms (CWDF) and classical bacteria.
3. There is evidence from this small series that bacteria in various forms may be present in the aqueous of patients with intraocular inflammation, whether that inflammation be evident clinically or whether it be occult.
4. Some correlation seemed to exist between the presence of those variant bacteria and certain findings in the history, the ophthalmological examination, the leukocytic cytology of the aqueous, and the peripheral blood smears of patients with uveitis.

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References*

1. Guze L: *Microbial Protoplasts, Spheroplasts and L-forms*. Baltimore, Williams & Wilkins Co, 1968
2. Smith L: *The Pathogenic Anaerobic Bacteria*. Springfield, Ill, Charles C. Thomas, 1975
3. Synder C: *Noguchi's granulosis, Our Ophthalmic Heritage*. Boston, Little, Brown & Co, 1961
4. Hessburg P, Mattman L, Barth C, Dutchshen L: Aqueous microbiology: The possible role of cell wall deficient bacteria in uveitis. *Henry Ford Hosp Med J* 17:177-193, 1969
5. Brown A: Chronic uveitis. Bacteriological and immunologic consideration. *Arch Ophthalmol* 12:730-750, 1934
6. Irvine R, Irvine M: A Study of aqueous humor as an aid to understanding inflammatory conditions of the eye. *Am J Ophthalmol* 25:150-163, 1942
7. Berens C, Rothbard S, Angevine D: Cultural studies on patients with uveitis and other eye diseases. *Am J Ophthalmol* 25:295-301, 1942
8. Verrey F: Bacteriologic de L'Humeur Aqueuse. *Bull Soc Ophthalmol Fr* 59:256-265, 1946
9. Von Sallmann L, Locke J, Locke B: Cytological and bacteriological studies of the aqueous humor in uveitis. *Arch Ophthalmol* 46:4-13, 1951
10. Matura H: Anaerobes in the bacterial flora of the conjunctival sac. *Jpn J Ophthalmol* 15:116-124, 1971
11. Luria D, Kaminski T, Grieco M, Singer J: Aberrant forms of bacteria and fungi found in blood or cerebrospinal fluid. *Arch Intern Med* 124:39-48, 1969
12. Moser R: Host factors: An overview. *JAMA*: 232(5)516-519, 1975
13. Kaufman H: Development of resistance in the eye, the secretory immunologic system. US DHEW, PHS, NIH, 1969, pp 325-329
14. Gorbach S, Bartlett J: Anaerobic infections (Parts 1,2,3). *New Eng J Med* 290(21):1177-1294, May 1974
15. Woods A: *Endogenous Inflammations of the Uveal Tract*. Baltimore, Williams & Wilkins Co, 1961
16. Cole R: Uveitis, review. *Surv Ophthalmol* 5:355-414, 1960
17. Allansmith M, Skaggs C, Kimura S: Anterior chamber paracentesis diagnostic value in postoperative endophthalmitis. *Arch Ophthalmol* 84:745-48, 1970
18. Amsler M: Bowman lecture new clinical aspects of the vegetative eye. *Trans Ophthalmol, Soc UK* 68:45, 1948
19. Offret G, Saraux H, Bisson J: Uveitis with presence of pathogenic bacteria in anterior chamber; results of 36 bacteriologic examinations of aqueous humor in 2 cases. *Bull Ophthalmol Fr* 66:321-337, 1953
20. Verrey F: Bacteriologic des Kammerwassers, Beitrag zur Aetiologie der Uvertis. (Bacteriology of the aqueous humor; with a contribution on the etiology of uveitis). *Klin Monatsbi Augenheilkd* 130(2):215-234, 1957
21. Offret G et al: Transplantation of fresh vitreous humor as a treatment of posterior uveitis. *Bull Soc Ophthalmol Fr* 68:23-27, 1968
22. Friedland B, Anderson D, Forster R: Non-lysozyme antibacterial factor in human tears. *Am J Ophthalmol* 74:52-59, 1972
23. Smith J, Israel C: Spirochetes in the aqueous humor in seronegative ocular syphilis. *AMA Arch Ophthalmol* 77:474-477, 1967
24. Goldman J, Girard K: Fluorescent antibody staining of treponemes in uveitis. *Arch Ophthalmol* 79:716-722, 1968
25. Simon C, Mollinedo R: Diagnostic de la syphilis par la recherche du granule spirochetogene. *Presse Med* 48:513-516, 1940
26. Yöbs A, Clark J Jr, Mothershed S, Bullard J, Artley C: Further observations on the persistence of *Treponema pallidum* after treatment in rabbits and humans. *Br J Vener Dis* 44:116-130, 1968
27. Klieneberger E: The natural occurrence of PPLO in apparent symbiosis with *Streptobacillus moniliformis* and other bacteria. *Path & Bact* 40:93-105, 1935
28. Mattman L: *Cell Wall Deficient Forms*. Cleveland, Chemical Rubber Co, 1974
29. Godzeski C: In vivo persistence of L-phase bacteria, in Guze L (ed): *Microbial Protoplasts, Spheroplasts and L-Forms*. Baltimore, Williams & Wilkins Co, 1967, p 379
30. Altemeier W, Hill E, Fullen W: Acute and recurrent thromboembolic disease: a new concept of etiology. *Ann Surg* 170:547-558, 1969
31. Kagan G, Koptelova E, Pokrovsky B: Isolation of pleuropneumonia-like organisms, L-forms and heteromorphous growth of bacteria from the cerebrospinal fluid of patients with aseptic meningitis. *J Hyg Epidemiol Microbiol Immunol (Praga)* 9:314-317, 1965
32. Sharp J: Mycoplasmas and bacterial L-forms: clinical considerations, in Madoff S: *Mycoplasma and the L-forms of Bacteria*. New York, Gordon & Breach Science Publishers, 1971, p 85
33. Kagan B: Antibiotic sensitivities of Staphylococcal L-forms, in Guze L (ed): *Microbial Protoplasts, Spheroplasts and L-Forms*. Baltimore, Williams & Wilkins Co, 1967, p 314
34. Green M, Heidger P, Dominique G: Demonstration of the phenomena of microbial persistence and reversion with bacterial L-forms in human embryonic kidney cells. *Infect Immun* 10:889-914, 1974

** Four hundred additional references are available from the author upon request.

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35. Hamburger M, Carlton J: Staphylococcal spheroplasts and L. colonies II. Conditions conducive to reversion of spheroplasts to vegetative staphylococci. *J Infect Dis* **116**:544-550, 1966
36. Kagan B: Staphylococcal L-forms: Ecologic perspectives. *Ann NY Acad Sci* **128**:81-91, 1965
37. Haenal H: Some rules in the ecology of the intestinal microflora of man. *J Appl Bacteriol* **24**:242-251, 1961
38. Rosebury T: *Microorganisms Indigenous to Man*. New York, McGraw-Hill, 1962
39. Pease P: Tolerated infection with the sub-bacterial phase of Listeria. *Nature* **215**:936-938, 1967
40. Isadienso O: Listeria sepsis and meningitis, a complication of renal transplantation. *JAMA* **234**(8):842-843, 1975
41. Tedeschi G, Amici D, Paparelli M: Incorporation of nucleosides and amino-acids in human erythrocyte suspensions: Possible relation with a diffuse infection of Mycoplasma or bacteria in the L-form. *Nature* **222**:1285-1286, 1969
42. Pohlod D, Mattman L, Tunstall L: Structures suggesting cell-wall-deficient forms detected in circulating erythrocytes by fluorochrome staining. *Am Soc Microbiol* **23**:262-267, 1972
43. Gelfand J, Bennet J: Active histoplasma meningitis of 22 years duration. *JAMA* **233**(12):1294-1295, 1975
44. Oishi M, Imai M, Takahashi T et al: Studies on bacterial L-form in ocular infections, report 2: Inoculation into rabbit eye. *ACTA Soc Ophthalmol, Jpn* **78**(5):201-204, 1974
45. Madoff S: *Mycoplasma and the L-forms of Bacteria*. New York, Gordon & Breach Science Publishers, 1971
46. Decker J: Proceedings of the conference of the relationship of mycoplasma to rheumatoid arthritis and related diseases. US DHEW, Washington, D.C., February, 1966
47. Pavan-Langston D: Mycoplasma in the etiology of anterior and posterior uveitis: an experimental model. *Arch Ophthalmol* **80**:416, 1968
48. Aronson S, Elliott J: *Ocular Inflammation*. St. Louis, The CV Mosby Co, 1972
49. Stewart H: Prolonged antibacterial activity of a fluorescein-anesthetic solution. *Arch Ophthalmol* **88**:385-387, 1972
50. Manual of tests for syphilis. US DHEW, PHS, Nat Commun Dis Center. Venereal Disease Program. Washington, D. C., US Government Printing Office, 1969
51. Blair J, Lennette E, Truant J: *Manual of clinical microbiology*. Amer Soc Microbiol, Bethesda, 1970
52. Emmons C: *Medical Mycology*. Philadelphia, Lea & Febiger, 1970
53. Brem A: Isolation of L-forms from clinical material. *Am J Med Technol* **35**:702-705, 1969
54. Dutchshen L, Hessburg P: A microtechnique for the isolation of cell wall deficient organisms from very small samples. *Henry Ford Hos Med J* **21**:1-12, 1973
55. King J, Theodore T, Cole R: Generic identification of L-forms by polyacrylamide gel electrophoretic comparison of extracts from parent strains and their derived L-forms. *J Bacteriol* **100**:71-77, 1969
56. Theodore T, Tully J, Cole R: Polyacrylamide gel identification of bacterial L-forms and Mycoplasma species of human origin. *Appl Microbiol* **21**:272-277, 1971
57. Dienes L: The morphology of PPLO and bacterial L-forms. *Ann NY Acad Sci*, **108**:375-379, 1963
58. Dienes L: Permanent stained agar preparation of mycoplasma and of L-forms of bacteria. *J Bacteriol* **93**:689-692, 1967
59. Truant J, Brett W, Thomas W Jr: Fluorescence microscopy of tubercle bacilli stained with auramine and rhodamine. *Henry Ford Hosp Med Bull* **10**:287-296, 1962
60. Beneke E: *Human Mycoses, Scope Monograph*. Kalamazoo, Mi, Upjohn Co, 1968
61. Schlaegel T: *Essentials of Uveitis*. Boston, Little, Brown & Co, 1969
62. Mankiewicz E: Mycobacteriophages isolated from persons with tuberculous and non-tuberculous conditions. *Nature* **191**:1416-1417, 1961
63. Mankiewicz E, Beland J: The role of mycobacteriophages and of cortisone in experimental tuberculosis and sarcoidosis. *Am Rev Resp Dis* **89**:707-720, 1964
64. Berger H, Zaldivar C, Chusid E: Anonymous Mycobacteria in the etiology of Sarcoidosis, *Ann Inter Med*, **68**:872-874, 1968
65. Collart P, Borel J, Duvel P: Significance of spiral organisms found after treatment in late human and experimental syphilis. *BR Vener Dis*, **40**:81-89, 1964
66. McKay K, Abelseth M, Vandremel A: Production of an enzootic-like pneumonia in pigs with "protoplasts" of Haemophilus para-influenzae. *Nature* **212**:359-360, 1966
67. Osmond D: The origins, life spans and circulation of lymphocytes, in Schwarz M, (ed), *Proc of the Sixth Leucocyte Culture Conference*, New York, Academic Press, 1970
68. Halpern B: Diagnosis of drug allergy in vitro with the lymphocyte transformation test. *J Allergy* **40**:168-181, 1967
69. Rebuck J: A method of studying leukocytic functions in vivo. *NY Acad Sci* **59**:757-805, 1955
70. Hessburg P, Rebuck J: Skin window responses in uveitis. *Am J Ophthalmol* **62**:648-659, 1966
71. Hogan M, Wood I, Godfrey W: Aqueous humor cytology in uveitis. *Arch Ophthalmol* **89**:217-220, 1973
72. Humphrey J, White R: *Immunology For Students of Medicine*. Philadelphia, FA Davis Co, 1963
73. Pavan-Langston D: Mycoplasmal anterior and posterior uveitis. I. Clinical manifestations of the experimental disease. II. Histopathologic manifestations of mycoplasmal uveitis. *Arch Ophthalmol* **82**:245-258, 1969
74. Clark E, Clark E: Observations on changes in blood vascular endothelium in living animal. *Am J Anat* **57**:385-438, 1935
75. Lancaster M, Allison F: Studies on the pathogenesis of acute inflammation. VII. The influence of osmolarity upon the phagocytic and clumping activity of human leukocytes. *Am J Pathol* **49**:1185-1200, 1966
76. Fritze E: Defence system of neutrophilic granulocytes. *Dtsch Med Wochenschr* **81**:601-604, 1956
77. Fleck L, Murczynska Z: Symposium of Polish medical contributions in World War II; leukergy; clumping of white blood cells in cytologically homogenous groups, new phenomenon of inflammation. *Tex Rep Biol Med* **5**:156-167, 1947
78. Monson R: Biologic false-positive FTA-ABS test in drug-induced lupus erythematosus. *JAMA* **224**:(7) 1028-1030, 1973
79. Yobs A, Olansky S, Rockwell D, Clark J Jr: Do treponemes survive treatment of late syphilis? *Arch Dermatol* **91**:379-389, 1965

80. Goldman J, Girard K: Intraocular treponemes in treated congenital syphilis. *Arch Ophthalmol* **78**:47-50, 1967
81. Christman E, Hamilton R, Heaton C, Hoffmeyer I: Intraocular treponemes. *Arch Ophthalmol* **80**:303-307, 1968 (Abstr Eye Ear Nose Throat Dig, March, 1969, p. 22)
82. Ustimenko L: L-forms of treponema pertenuae, in Guze L (ed): *Microbial Protoplasts, Spheroplasts and L-forms* Baltimore, Williams & Wilkins Company, 1967
83. Weber J, Schlaegel T, Golden B: Statistical correlation of uveitis syndromes with virus titers. *Am J Ophthalmol* **78**(6):948-951, 1974
84. Smith P: The possible role of bacterial L-forms in disease. *SD J Med* **19**:40-45, 1966
85. DeSchweinitz G: Choroidal diseases, their relation to general diseases and particularly to infections, intoxications and auto-intoxications. *Ann Ophthalmol* **15**:498-517, 1906
- 85a. Billings F: *Focal infection*. In the Lane Medical Lectures. New York, Appleton, 1916
86. Clasener H: Pathogenicity of the L-phase of Bacteria. *Am Rev Microbiol* **26**:55-84, 1972
87. Bertalanffy L, Masin M, Masin M: Use of acridine orange fluorescence technique in exfoliative cytology. *Science* **124**:1024-1025, 1956
88. Lynn R, Morton H: The inhibitory action of agar on certain strains of pleuropneumonia-like organisms. *Appl Microbiol* **4**:399-341, 1956
89. Spaar F, Bauer H: Demonstration of tumor cells in cerebro-spinal fluid by fluorescence microscopy with acridine orange. *Zeiss INFO* 31-34, 1970
90. O'Beirne A: CWD forms of Listeria produced by growth in lung macrophages were never revertable. PhD Thesis, Univ Of Michigan, Dept Public Health, 1973
91. Calderon N, Albuerno A, Gonzalez S, Winkler L: Cell wall deficient bacteria (L-forms) in meningitis. *Lat Am Microbiol*, **13**:95-100, 1971
92. Carvill L: Tubercular iritis. *Am J Ophthalmol* **3**:742, 1921
93. Zweifach B, Grant L, McCluskey R: *The Inflammatory Process*. New York & London, Academic Press, 1965
94. Hui L, Tien-Lin L, Ai-te L: The L-form of Proteus vulgaris. II. Cytochemical study of L-form of Proteus, using Feulgen's staining method and fluorescence microscopy with acridine orange. *Sci Sinica* **13**:1829-34, 1965
95. Altenbern R: Critical factors influencing growth of L-forms of P. mirabilis. *J Bacteriol* **81**:586-594, 1961
96. Medill M, O'Kane D: A synthetic medium for the L-type colonies of proteus. *J Bacteriol* **68**:530-533, 1954
97. Mattman L: L-forms isolated from infections, in Guze L (ed): *Microbial Protoplasts, Spheroplasts and L-forms*. Baltimore, Williams & Wilkins Company, 1967, p 472
98. Columbia team links bizarre pathogen to idiopathic uveitis, rheumatoid arthritis. *Clin Trends* **13**:6, 1975
99. Hessburg P: Management of Pseudomonas keratitis. *Surv Ophthalmol* **14**:43-54, 1969
100. Zieve P, Haghshenass M, Blanks M, Krevans J: Vacuolization of the neutrophil: an aid in the diagnosis of septicemia. *Arch Intern Med* **118**:356, 1966
101. Steigbigel R, Johnson P, Remington J: Nitroblue tetrazolium reduction test vs conventional hematology in diagnosis of bacterial infection. *New Eng J Med* **290**:235-238, 1974
102. Barth CL: "Studies on the Etiology of Uveitis: Microbiology, Immunology, Hematology." Ph.D. Diss., Wayne State Univ., 1974.