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Studies on uveitis Part I: Aqueous studies

Philip C. Hessburg, MD*

Aqueous microbiological and cytological studies were performed on 134 anterior chamber taps done on 118 patients both with and without uveitis. Cell wall defective bacterial forms (CWDF) were recovered from about 40% of these aqueous specimens and classical bacteria were recovered from 12%. A statistically significant difference could not be found between the incidence of bacterial cell wall defective forms in the various anatomical types of uveitis. There were hints from this work that the presence of CWDF in the aqueous was more frequent in younger patients with uveitis, early in the uveitis attack, if there were multiple foci of infection, or if there was 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. The slit-lamp presence of many cells, dense aqueous flare, or of hypopyon appeared significant, as did the presence of mutton fat KP. The presence of aqueous CWDF seemed to be related to the presence of certain leukocytes.

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Introduction

THE purpose of these studies was to learn whether bacterial cell wall defective¹ forms or either aerobic or anaerobic² classical bacteria are found within the eye, and if so, whether these microorganisms occur more frequently in active uveitis. Improved microbiological techniques were used to culture fastidious microbiological organisms from the aqueous of patients both with and without uveitis. Simultaneous studies correlated the presence of microorganisms with cytological factors in the aqueous and with systemic hematological, immunological, and serological factors.³

The possible presence of bacterial cell wall defective forms (CWDF) in the aqueous has been the subject of only one known study,⁴ although others have been done on the incidence of classical bacteria in the aqueous.⁵⁻⁹ With improved microbiological techniques, it may be possible to recover forms of bacteria not previously known to exist there. These techniques include more complex media, anaerobic techniques,¹⁰ fluorescent staining, microscopic examination of cultural media, prolonged incubation periods, microtechniques adapted from tissue culture laboratories, and others.

There are many reasons why traditional microbiological laboratories will almost invariably fail to uncover fastidious organisms from chronic or relapsing disease states:

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1. They discard smears which do not display classical bacterial morphology and consider as artifactual any amorphous gram-negative material. (Cell wall defective bacteria are amorphous and stain gram-negatively.)
2. They discard plates or broths which do not show growth within seven days. (Many fastidious cell wall defective bacteria, especially clinical isolates, do not make their presence known for 30 to 60 days.)
3. They only rarely use more than two standard media for all clinical specimens. (Common artificial and natural media, especially those containing agar, are known to have a suppressing effect on cell wall defective forms. Often, as many as seven different media may be required to find one on which a fastidious organism will survive.)
4. They examine plates and broths by gross observation. (The small size of the colonies of many cell wall defective bacteria are visible only with a microscope, and the slit lamp, using Tyndall effect, is often needed to observe colonies or wispy growth in liquid media.)
5. They assume that any bacteria of importance can be repeatedly subcultured. (The nutritional requirements of cell wall defective bacteria are often poorly understood, and growth which appears luxuriant may be lost on subculture.)
6. They assume that growth will be found on the surface of plates. (Fastidious organisms often grow into solid media rather than on the surface in a spreading fashion.)
7. They fail to utilize serial subculture techniques. (Often, fastidious organisms can only be recovered by initial inoculation into tiny quantities of fresh media followed by frequent serial transfer into larger quantities of the same fresh media.)
8. They generally cling to stereotyped conditions. (Many fastidious microbiological forms require individualized techniques, i.e., serum of one sort or another, anaerobic conditions, serial subcultures, and carefully controlled hypertonicity to shield the wall defective form.)

The studies of aberrant bacterial and fungal infections,¹¹ variations in host responses,^{12,13} synergistic bacterial infections, or poly-microbial infections and of mixed aerobic-anaerobic infections are in their infancy, especially in ophthalmology. One need only to remove fluid from an eye with postoperative hypopyon and receive a laboratory report of "sterile pus" to suspect the truth of Gorbach and Bartlett's statement that "sterile pus" is "usually a refuge for bacteriologic ineptitude".¹⁴

For many reasons then, it is not surprising that most investigators have considered the aqueous sterile. Woods defined non-granulomatous uveitis as a "sterile non-purulent inflammation resulting from a probably toxic or allergic insult to the tissue" and noted that "all the bacteriological work we have points to the sterility of these eyes."¹⁵ He felt that if organisms did arrive in the eye they were dead on arrival, phagocytosed quickly, or owed their pathogenicity to antigenic or toxic rather than infectious properties. Other early investigators of uveitis also suggested that the aqueous was sterile.

Coles¹⁶ stated that "the absence of bacteria in the aqueous during the attacks of iridocyclitis (anterior uveitis) seems to be well established in this country." The utilization of aqueous humor cultural techniques is only rarely advocated, and then only when a postoperative infection is suspected.¹⁷ Such has not been the case in Europe where a microbial link has been more thoroughly sought and more often found.¹⁸⁻²⁰ Actually, at one time or other, most organisms have been isolated from the aqueous.

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Nevertheless, to explain this supposed aqueous "sterility", it has been suggested that aqueous might be self-sterilizing or have bactericidal or antimicrobial activity.²¹⁻²³ Could the antimicrobial properties of the aqueous humor strip the cell wall from classical bacteria? This possibility suggested that improved microbiological techniques could be used to study the aqueous. Although the literature associating bacterial CWDF and uveitis is particularly sparse, it is intriguing, in view of the currently great interest in syphilis,²⁴ that the spirochete does occur as a CWDF.²⁵ Yobs²⁶ has found that treated patients can still harbor *Treponemes*, in which case penicillin must have been only bacteriostatic and could have induced the CWDF of *Treponema pallidum*.

Kolmer was among the first to suggest that nongranulomatous uveitis was an allergic disease. Many have suggested that uveitis is produced by an allergic relationship between various bacteria and the eye. Iritis, commonly considered a hypersensitivity phenomenon of the iris to a variety of assaults, has been associated in this way with many bacteria.

Classical bacteria and CWDF

The classical bacterium has a rigid cell wall which accounts for its characteristic shape and staining characteristics. The cell wall is not necessary for life, however, and it is now known that virtually all bacteria familiar to ophthalmologists (with the possible exception of *Toxoplasma gondii*) can exist in a cell wall defective form.

A cell wall defective bacterium has shed part or all of the rigid wall to become an amorphous, shapeless, protoplasmic bag delimited only by a cell membrane. Considerable confusion exists in terminology. Therefore, under the generic term "bacterial cell wall defective form (CWDF)", we group the terms L-form, spheroplast, granule, large body, protoplast, fastidious bacterial variant, and microplast. The bacterial L-form was

first described by Klieneberger-Noble²⁷ and was named "L-form" in honor of the Lister Institute where she worked. These bacteria are larger than viruses although smaller than most classical bacteria, averaging 300 to 1000 nm in size. When grown on solid media, the colonies are usually extremely tiny and granular. When L-forms are created by antibiotics *in vitro*, the colonial morphology is often of the "fried egg" variety, but this configuration is rarely seen when the organisms are isolated from *in-vivo* clinical sources. Without a complete cell wall, the bacterial L-form has a nonrigid exterior surface, which accounts for marked pleomorphism. Without a cell wall, traditional bacterial stains become useless, as all cell wall defective forms look gram-negative).

Protoplasts can be defined as bacteria reduced to a cell membrane by complete stripping of the cell wall and requiring a hypertonic medium to survive. This is usually accomplished in the laboratory by including hypertonic sucrose in the media. Spheroplasts possess some cell wall but are also fragile and, like protoplasts, stain as gram-negative spheres.

The wall defective bacterial forms come from a parent classical bacterium which has been stripped from its cell wall by antibiotics,²⁸ hypertonicity, lysozyme, antibody or other unfavorable conditions. This process, known as conversion, is easily produced in the laboratory and can occur *in vivo*. The natural sequence of events is for the host to strip the cell wall from an invading bacterium prior to phagocytosis. Sometimes, however, the bacterium can exist in this cell wall defective state either as an intracellular parasite or in any body fluid where tonicity is suitable.

Absence of the cell wall offers a tremendous advantage to the bacterium for retaining viability. Antibiotics which are active at the cell wall become ineffective, and the organism can slip into a form so unrecognizable to the host immune mechanisms that

it may exist in tissue or within cells for prolonged²⁹ periods. If those forces which initially stripped off the cell wall cease to exert an influence, the potential pathogen may reclothe itself in a cell wall, that is, "revert" to a classical bacterium and reassume pathogenicity. The cell wall defective state is, however, a preliminary stage in the body's defense mechanisms against the bacterium and must occur before complete phagocytosis can take place (Figure 1).

The cell wall defective bacterial state is known to play a role in disease pathogenesis.^{30,31} Formerly considered nonpathogenic by virtue of their lack of an antigenic cell wall, CWDF are now known to have pathogenic capability even if they have no cell wall. Coagulase-positive *Staphylococci* in their variant form retain coagulase activity. It has been learned that *Clostridium tetani* and *Clostridium botulinum*, completely free of cell wall, still make toxins, and that wall-free *Staphylococcus* continues to produce common Type A Enterotoxin. This is true also for *Candida*, *Listeria*, *Meningococcus*, etc. Such pathogenic functions do not depend on a cell wall. The staphylococcal and streptococcal CWDF have been the sole isolates from a variety of diseases.^{32,33}

In general, CWDF made *in vitro* are less apt to be virulent than ones occurring spontaneously *in vivo*. However, many test-tube-produced variants have also been found pathogenic for experimental animals.

Once converted to the cell wall defective form, the variant phase may be "stabilized" by another group of compounds. Most *in vitro* stabilizers are the simple salts of inorganic cations, such as magnesium sulfate. In situations which would otherwise be osmotically disruptive, they accomplish this by increasing the tonicity of the medium itself and by biochemically strengthening the cell membrane to withstand increased osmotic pressure. This stabilization process can be so effective that some CWDF will not revert to

the classical form by any known means and are known as "stable L's" or "nonrevertable L's". Green, Heidger, and Domingue³⁴ have shown that the L-forms of *Streptococcus faecalis* can have variable stability. In experiments using human embryonic kidney fibroblasts, they infected these cells with either "relatively stable" or "stable" L-forms of this organism. The relatively stable L-forms could be cultured from the infected cells for only about one week following inoculation. The microorganisms would then revert. At the time of reversion, both L-forms and transitional variants, plus intra- and extracellular bacterial forms could be found by electron microscopy. Stable L-forms, on the other hand, were culturable throughout the experimental period (as late as 73 days after infection), and although these L-forms underwent morphological changes within the kidney cells, they could be found intracellularly for long periods.

At any time, the cell wall defective phase may revert to the classical bacterium, a process known as reversion.³⁵ Unfortunately, there are as yet no foolproof methods to stimulate such reversion. Sometimes the removal of penicillin or blood serum from the media, the addition of yeast, or switching from liquid to solid media will induce reversion. Kagan stated that L-forms (CWDF) "do what they want to do" regarding reversion.³⁶ Various media have been suggested, but a consistent method of reverting clinical isolates is unknown. When the various host factors which normally suppress bacteria *in vivo* are somehow altered, the cell wall defective bacteria, which may be in a less pathogenic form, can recover full pathogenicity by reversion to the classical bacterial state.

Bacteria and CWDF in the eye

Haene³⁷ stated that "the macroorganism and its microflora form a balanced ecological system." The tremendous diversity of the human microflora has been noted in the compendium of aerobes prepared by Rosebury³⁸, and our anaerobic microflora has

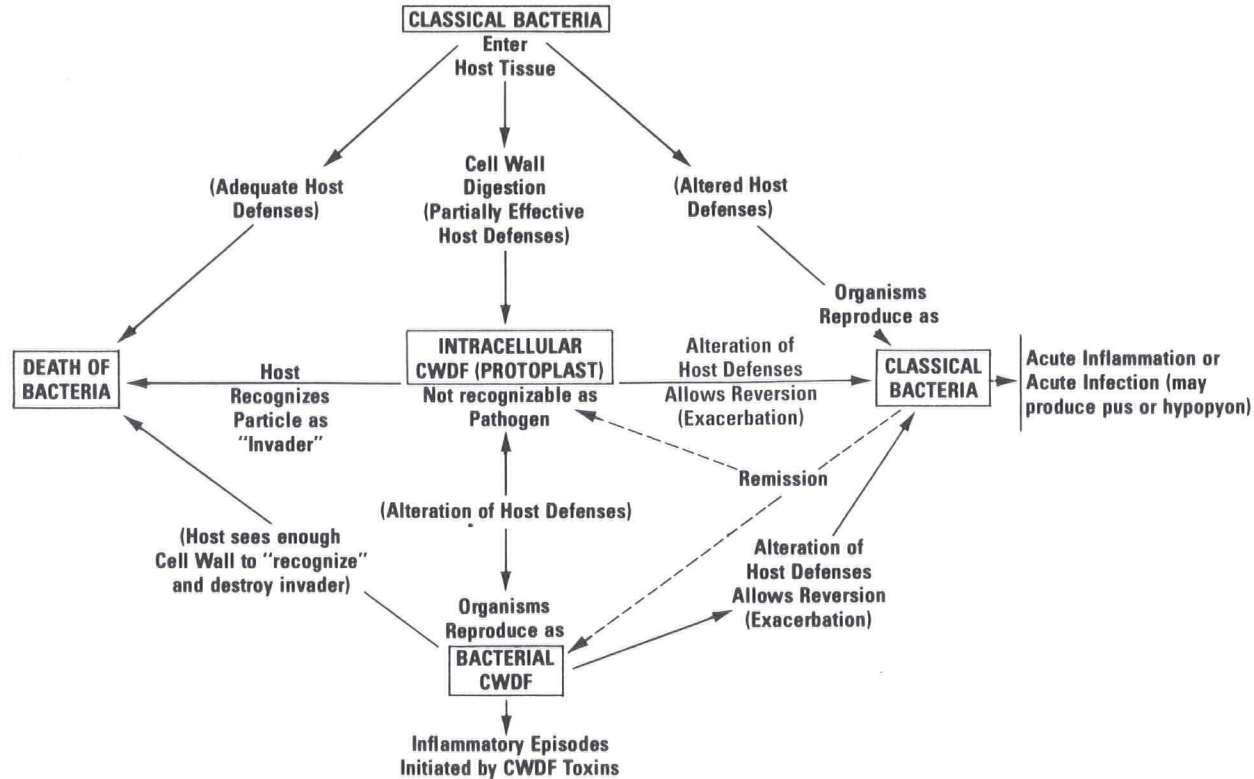


Figure 1

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.³² Inflammatory episodes either in response to bacterial forms or their toxins may occur with alterations in the host defenses in several ways.

been carefully documented in a recent monograph of Smith.² Matura¹⁰ has studied the microflora of the conjunctival sac, recovering aerobes from 95% of people and anaerobes from 80%. Normal "uninfected" tissues can harbor bacteria.^{28,38} Postmortem studies reveal that almost 60% of autopsy tissues contain bacteria and/or fungi other than *Mycobacteria* or virus. An abundant literature documents the presence of classical and aberrant *Listeria*.^{39,40} Tedeschi⁴¹ has described an organism found in the red cells of man which shows no cell wall by electron microscopy. Pohlod⁴² found cell wall defective bacterial forms in the erythrocytes of 57% of control persons.

The interior of the eye houses encysted *Toxoplasma gondii* for decades, *Histoplasma capsulatum* presumably for years, *Spirochetes* for decades, and some viruses for a lifetime. It is possible that the eye may also house common pathogenic bacteria in a variety of states, just as it is known to house other pathogenic microbial forms, such as those mentioned above. The bacterial species of most common interest in ophthalmic pathology are known to have a cell wall defective form. The relationship of the role which might exist between such fastidious microbiological forms and intraocular inflammation is completely unknown.

Perhaps classical bacteria should not be expected in chronic or relapsing diseases such as uveitis. As soon as antibody-laden secondary aqueous becomes available, pathogenic bacteria should be expected either to undergo phagocytosis, to lose their cell wall, or to adopt an intracellular hiding place. Only in overwhelming infections, such as an infected corneal ulcer, would many organisms be expected to exist in a classical state. Large numbers of organisms must not be expected in chronic or relapsing situations where the host mechanisms and the invading systems are closer to an ecological equilibrium.

Although bacterial infections generally run a fairly predictable course, such is not

always the case. Recently a patient was described⁴³ with active *Histoplasma capsulatum meningitis* of 22 years' duration. During this time cerebrospinal fluid cultures were positive on five occasions and negative on five others. Obviously, the host patient and his invading organism had reached a stalemate, and, just as obviously, it must be assumed that at the time of negative cerebrospinal fluid cultures the organism remained hidden but viable in some other tissue.

The only known study of the intraocular pathogenicity of bacterial L-forms in experimental animals is that of Oishi.⁴⁴ He inoculated the anterior chambers or conjunctival sac of rabbits with the L-forms and parent classical organisms of a *Staphylococcus epidermidis* (isolated from a case of chronic catarrhal conjunctivitis) and a strain of *Corynebacterium*. He noted no irritation or inflammation in either the conjunctival sac or the anterior chamber of animals inoculated with the CWDF of the species, but when the classical bacteria were injected into the anterior chamber, the animal developed an acute anterior uveitis. This experiment proved only that, under these circumstances, the healthy rabbit was not sensitive to the CWDF of these two bacterial species. It cannot be interpreted as meaning that the same rabbits, if prestressed with cold, steroids, or other resistance-altering physical or chemical stresses, would have reacted similarly. Godzeski (personal communication) has noted that when 1 ml of *Salmonella* "L"s, concentrated to a slurry of 10^{13} organisms per ml, are injected into the bloodstream of a normal mouse, they will completely disappear from the bloodstream within 30 minutes. The normal reticuloendothelial system apparently is able to dispatch them completely. However, if the animal is stressed by subjecting it to intense cold before injection or by giving it steroids, then the L-form of the *Salmonella* can be recovered for many months thereafter. Godzeski believes that the healthy, normal animal uses well-established defense mechanisms to clear out organisms which

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the stressed or abnormal animal cannot call into play.

Although there is a close resemblance⁴⁵ between bacterial CWDF and *Mycoplasma* in growth characteristics, fine structure, media needs, etc., mycoplasma have not been isolated from uveitis. Formerly called PPLO, mycoplasma are small organisms (125 to 220 nm) which classically grow out as fried egg colonies on solid media. Classified in the microbiological order *Mycoplasmatales*, they grow extracellularly, lack a cell wall, reproduce by budding and by extension of fine filaments. *Mycoplasma* never revert to classical bacteria. They have been recovered from a variety of sources⁴⁶ and are known to cause disease in animals and man. There have been multiple isolations of mycoplasma from the ocular adnexal tissue of animals and man. Pavan-Langston⁴⁷ has shown that *Mycoplasma pulmonis* can cause uveitis in rabbits, (mycoplasma have been found in the prostatic and vaginal secretions of patients with uveitis), but studies searching for mycoplasma in iritis have consistently been negative.

Traditional microbiological thinking is based on a concept of "microbial monoetiology". Following the Pasteur-Koch-Ehrlich format, the principle of "one microbe—one disease—one drug" has become dogma. Gorbach and Bartlett state that "the concept of monoetiology applies to infections such as lobar pneumonia, typhoid fever, diphtheria, and cholera. But this classic design does not fit most of the infections associated with anerobic bacteria. These septic processes harbor multiple strands of microorganisms with varying oxygen sensitivities and undefined pathogenic potentials."

The fact that much of this work was carried out in an anaerobic, gas pack jar system suggests that some of the organisms recovered may have been aerotolerant and moderately obligate anaerobes—especially

those transitional forms with unknown nutritional requirements.

Thus, although a concept of the etiology of uveitis still escapes us,⁴⁸ there is a considerable literature which points to a possible relationship between uveitis and almost every known microorganism, either directly or on some sort of hypersensitivity basis.

Materials and methods

A total of 134 anterior chamber taps (ACT) was done on 118 people. Results are shown in Tables I and II. A specimen of blood was obtained from the last 50 patients at the same time as the anterior chamber tap was performed.

Four other nonaqueous specimens (two specimens of cerebrospinal fluid, one each of vitreous and uveal tissue) were studied by the same microbiological techniques used for the aqueous specimens.

The anterior chamber tap was made through a small limbal area prepared with an alcohol-soaked pledget. Anesthesia was obtained with sterile cocaine rather than with commercially available corneal anesthetics since the latter contain germicidal agents⁴⁹ whose effects on the aqueous are not fully known. For the same reasons, exhaustive conjunctival sterilizing procedures were not performed since such techniques might alter the flora of the anterior chamber as well as of the conjunctiva.

In an operating room, aqueous was obtained by using a sterile, disposable one-inch 27 gauge needle on a sterile, disposable tuberculin syringe. About 0.20 ml of the aqueous was usually withdrawn.

Inoculation was always made onto sheep blood agar to recover *Saprophytes* or bacteria from the conjunctival sac.

Nonmicrobiological aqueous studies performed were a darkfield test for *Spirochaeta*,

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an anticomplement test for *Spirochaeta*,⁵⁰ and a Leishman's stain for cytologic detail.

Microbiological studies were performed on the aqueous with progressively more complex laboratory regimens during four distinct, successive phases.* Studies in the first phase used solid media which had been subcultured from two liquid media tubes of thioglycollate broth and Medill-O'Kane broth which were inoculated at the time of anterior chamber tap. After 5 days, and again after 30 days, each of these was subcultured into Sabouraud's maltose agar, Chanock's solid agar, sheep blood agar, and three TB media (American Trudeau Society (ATS) medium, Petragnani medium and Lowenstein-Jensen medium).

During the second phase, seven liquid media were used for the initial inoculation. Each was subcultured at five and 30 days to Chanock's solid agar and sheep blood agar plates. The initial media were Thioglycollate (Brewer's Broth), Mattman's Thioglycollate Broth "X", Medill-O'Kane (Tunstall) Broth, Kinsey's Kresge Eye Institute Medium #4 without cholesterol, Kinsey's Kresge Eye Institute Medium #4 with cholesterol, Mattman's Veal Heart Infusion Broth and Kirschner's Broth.

In the third phase, each of the media used in the second phase, along with Beef Heart Infusion Broth, was serially subcultured on the 5th, 10th, and 15th day to fresh tubes of the same medium. On the 5th day, each was also subcultured to a tube of veal heart infusion broth. On the 20th day, each of

these tubes was again subcultured to fresh tubes of the same media, to a veal heart infusion tube, and onto plates of sheep blood agar, "L" agar and Chanock's agar (Figure 2).

During the second, third and fourth phases, Sabhi medium was substituted as an initial isolation medium for fungi and Kirschner's medium substituted for other TB media. Each of these changes was made to enhance the possibility of isolating the respective CWDF.

In the fourth and most recent phase, a microwell technique⁵⁴ made it possible to inoculate a wide variety of initial media and still lessen the dilution effect seen when a very small inoculum is put into a 5 cc screwtop macro-tube. The same media used in phase three were placed in side-by-side tissue culture microwells. One of each pair was then inoculated with a drop of aqueous; the other served as an uninoculated control. At three days, each microwell was subcultured to a macro-tube of fresh medium and at seven days subcultured to another tube of the same medium and to a tube of veal infusion broth. At ten and fifteen days, each tube (except for veal infusion tubes) was again subcultured to fresh media. On the 20th day, tubes were again subcultured to tubes of the same fresh media, to tubes of veal infusion broth, and to plates of sheep blood agar, "L" agar and Chanock's media.

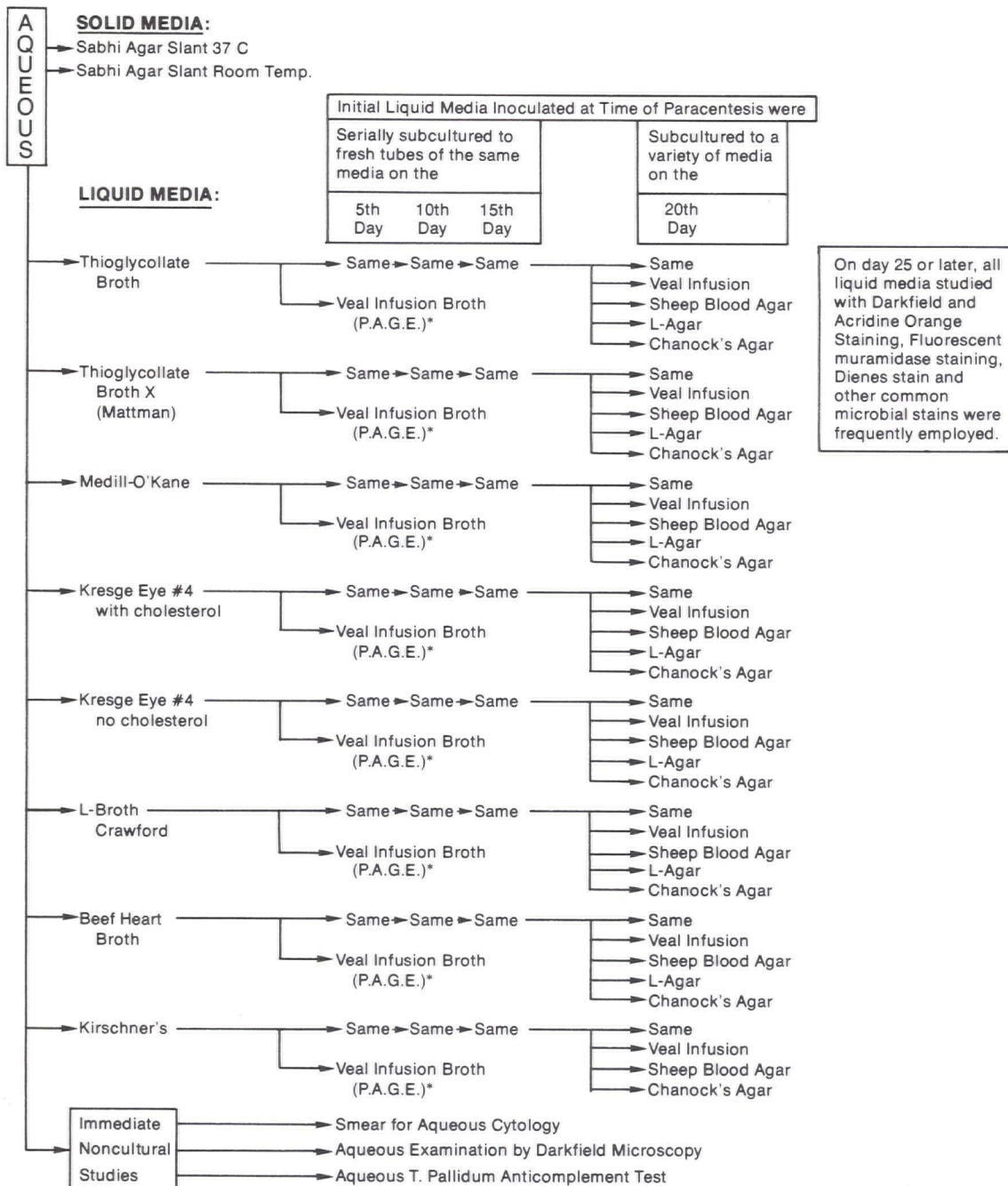
When classical bacterial colonies were seen on solid media, they were carved out and subcultured in thioglycollate broth to facilitate identification by standard microbiological techniques.

Tubes and plates were subcultured anaerobically in GasPak®* jars at 37 C except for one Sabourauds or Sabhi tube which was incubated aerobically at room temperature. Both solid plates and liquid media were

* Media for this study were chosen to isolate classical bacteria⁵¹ (thioglycollate and sheep blood agar), fungi⁵² (Sabouraud's agar and Sabhi) cell wall defective forms,^{28,53,96} mycoplasma, and an attempt was made to isolate *Spirochaeta* or other fastidious organisms on artificial aqueous (Kinsey's media). Inoculation on solid plates was performed within an area demarcated on the undersurface of the solid media plate by wax crayon. The surface of the agar was broken and furrowed with the inoculating glass rod to facilitate growth of cell wall defective bacteria into cracks in the media.

* BBL GasPak® Anaerobic Systems, Registered Trademark, Becton, Dickinson and Co, Cockeysville, Md

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* Large Veal Infusion broth tubes were constantly agitated by sterilized magnets to foster growth in quantities sufficient for Polyacrylamide gel electrophoresis.

Figure 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.

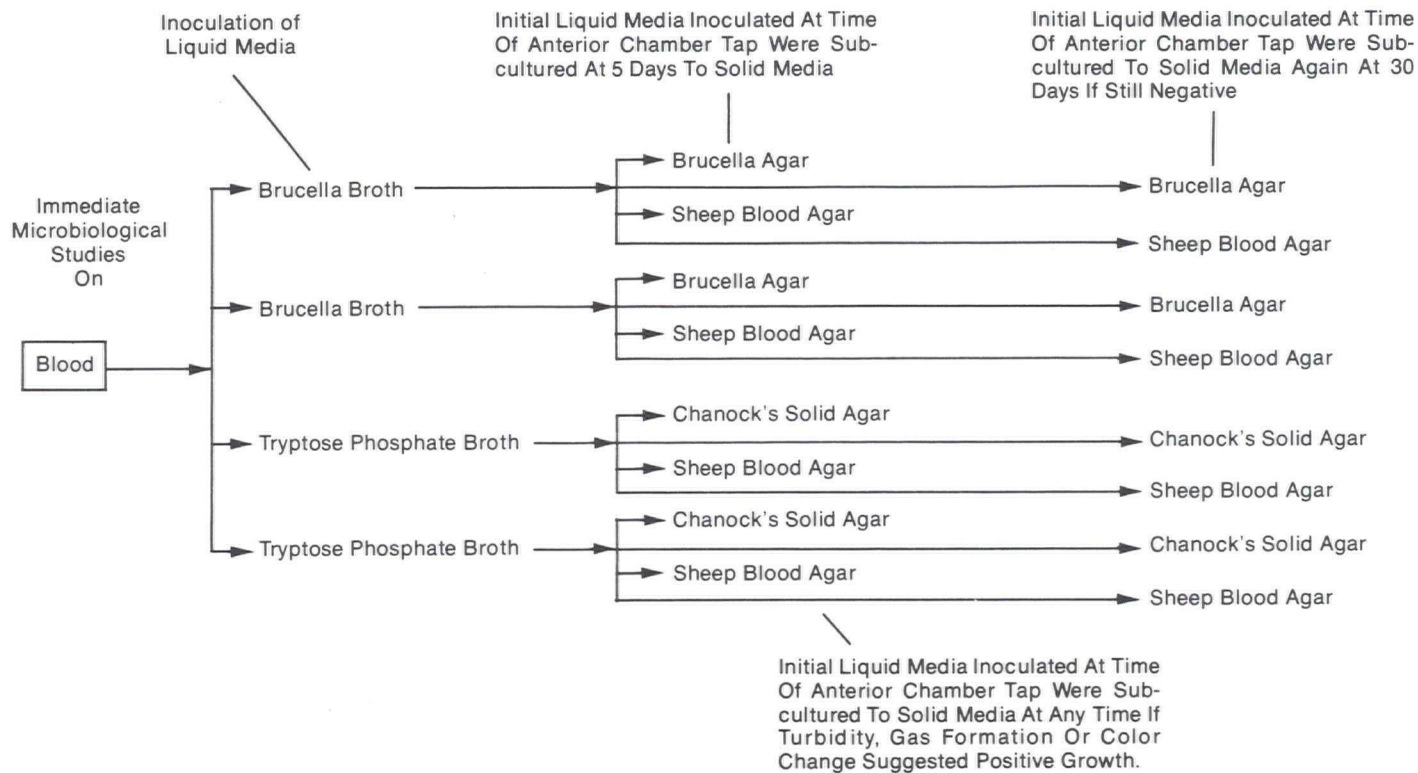


Figure 3
Microbiological studies performed on the blood of the fifty most recent patients are diagramed on this flowsheet.

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examined weekly. None were discarded before 60 days, and some materials were incubated as long as 180 days.

No isolation of a bacterial CWDF was considered positive until it had occurred on two or more different media, all controls of which remained negative. (Isolations which occurred on only one medium will be noted.) Confirmation of suspected cell wall defective forms was with acridine orange staining, sometimes by^{55,56} polyacrylamide gel electrophoresis, sometimes by reversion.**

Suspected areas of growth of solid agar plates were often stained with Dienes stain.^{57,58} Even with this stain, cultural patterns observed using 40X light microscopy or slit-lamp illumination were often not visible to the naked eye. Suspicious areas of solid media were carved out of the agar with sterile instruments, push-blocked across sterile, alcohol-flamed glass slides, and then stained. Such push-blocks of solid media or loopfuls of liquid media were stained with acridine orange or other stains and studied under white light or fluorescent microscopes.

Growth on TB media was usually stained with both acid-fast stain and with auramine-rhodamine (Truant) stain.⁵⁹

** In a laboratory manual to be published in the near future, details will be presented for the preparation of the 19 media used in this study. The four fluorescent stains and other cytological and microbiological identification and speciation stains, along with details of unusual microbiological techniques such as push-block staining, preparation and sterilization of micro-wells, agar inoculation and surface furrowing techniques, media examination by microscopy or by slit-lamp with Tyndall effect, and techniques for constant agitation of liquid media, crayon marking of agar plates will also be presented. This manual for the isolation of cell wall defective forms from aqueous will also illustrate serial inoculation techniques and some preliminary techniques for polyacrylamide gel electrophoresis.

Gram stain, methylene blue, and India ink were used for fungal morphology.⁶⁰

Histories were taken and eye examinations performed but no attempts were made to do a complete uveitis survey. Concomitant blood studies were done on most patients.

The 50 specimens of blood which were studied microbiologically were obtained during the latter two phases of the aqueous studies. The microbiological plan used on these blood specimens is outlined by diagram in Figure 3.

Results

As Table I shows, 118 patients who had had a total of 134 anterior chamber taps were divided according to the anatomical site of their uveitic inflammation or the absence of uveitis. Findings of classical bacteria and bacterial CWDF in aqueous and blood are shown on Table I. Other data are on Table II. In this series and most others where patients with varied types of uveitis are compared, anterior⁶¹ uveitis occurs more frequently than posterior (Table I). It was surprising when almost 1/5 of the total uveitis group (16 patients) had evidence of generalized uveitis. This probably represents desperation on the part of the clinicians who settled for a "diagnostic" anterior chamber tap as a reasonable action in the most severe cases.

Bacterial cell wall defective forms were recovered from 50 of 124 aqueous specimens. (Laboratory accidents occurred with nine specimens, and with one, no microbiological studies were performed.) As the table shows, CWDF were recovered from 41% of patients with anterior uveitis, 36% of those with posterior uveitis, 35% of those with generalized uveitis, and 43% of those without uveitis.

Classical bacteria were eventually cultured from the aqueous of 12% of these aqueous specimens. Many of the classical

bacteria were probably reverted bacterial CWDF.

There was no relationship between the presence of classical bacteria or bacterial cell wall defective forms and the anatomical variety of uveitis. In fact, classical bacteria and bacterial cell wall defective forms can be isolated from the aqueous of patients who have no ocular inflammation.

In addition to the aqueous microbiological studies, simultaneous acceptable microbiological studies were performed on the blood of 46 patients, with CWDF and classical bacteria recovered as shown on Table I. Highly enriched media held in culture for long periods produced higher numbers of positive blood cultures both for CWDF and for classical organisms than are generally found in routine clinical microbiology laboratories. The numbers of positive isolations, however, are not higher than those found by others working with similar techniques in this field.^{28,42}

Associations or trends related to the anatomical variety of uveitis

As Table II shows, the most severely involved patients, those with generalized uveitis, were also the youngest, 75% of them under 45 years old. Patients with anterior uveitis also tended to be younger than those with posterior uveitis. The absence of uveitis in older patients can be explained by the fact that many such patients had an anterior chamber tap prior to a routine surgical procedure such as for cataract extraction, retinal detachment, etc.

There is evidence to suggest that CWDF are an intracellular guest during periods of inactivity. Thus, even if CWDF play a role in uveitis, they might not be recovered from the aqueous if the disease were inactive.

Patients with anterior uveitis come to early attention because of pain and photophobia. Within 72 hours of onset of the attack, an

anterior chamber tap was performed on 18% of the patients with anterior uveitis. No other patients had an anterior chamber tap sooner than in three days. Posterior uveitis tends to be seen later, since, without pain, patients often wait for spontaneous recovery of vision. In 90% of patients with posterior uveitis, the anterior chamber tap was not done until the attack had been underway for over one week. Patients with generalized uveitis also tend to smolder for long periods; over half had no paracentesis until the attack had been in progress for over one month.

Patients with posterior uveitis tended to have had fewer recurrences than those with anterior or generalized uveitis. No patient with posterior uveitis had had more than five previous attacks, while 35% of patients with anterior uveitis had had six or more previous attacks.

A history of "arthritis" occurs more commonly in patients with anterior than with posterior uveitis, as the figures show.

A substantial literature^{62,63,64} suggests the CWDF play a role in sarcoid. Five patients in this series had sarcoid established by biopsy; one of them had both anterior and posterior uveitis. The incidence of diabetes mellitus (10%) was higher than expected in the general population.

The high incidence of syphilis in our group could reflect a general increase in venereal disease or be due to the hospital's urban location, but it is probably due to a desire on the part of the investigators to isolate *Spirochaetae* from patients with a history suggestive of syphilis. A history of syphilis was reported most among patients with general uveitis (31%) and those with posterior uveitis (27%). The lowest incidence was among those with anterior uveitis (7%).

Multiple sclerosis is presently thought to be a "slow virus" disease. There is no evidence in the literature that CWDF play a

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TABLE I
MICROBIOLOGICAL FINDINGS RELATED TO THE
ANATOMICAL VARIETY OF UVEITIS

	Anterior Uveitis	Posterior Uveitis	Generalized Uveitis	No Uveitis	Totals
Number of Patients	60 (69%)	11 (13%)	16 (18%)	31	118
Number of Anterior Chamber Taps	68	11	21	34	134
Acceptable Microbiological Aqueous Studies	63	11	20	30	124
Bacterial CWDF in Aqueous	26 (41%)	4 (36%)	7 (35%)	13 (43%)	50 (40%)
Classical Bacteria in Aqueous	5 (8%)	2 (18%)	2 (20%)	2 (20%)	15 (12%)
Acceptable Microbiological Blood Studies	23	4	8	11	46
Bacterial CWDF in Blood	3 (13%)	1 (25%)	3 (37%)	4 (36%)	11 (24%)
Classical Bacteria in Blood	4 (17%)	1 (25%)	1 (12%)	0 (0%)	6 (13%)

role. Anterior chamber taps were performed on three patients with multiple sclerosis, one with anterior uveitis, one with generalized uveitis, and one without uveitis.

None of the patients had a history of "presumed" histoplasmosis. Five of six patients with a history of a diagnosis of toxoplasmic uveitis had posterior uveitis; the sixth had generalized uveitis.

Almost all patients with active anterior uveitis or with generalized uveitis had evidence of cells in the anterior chamber by slit lamp. Cells were never seen in eyes with no uveitis and in only 54% of those with posterior uveitis. Both anterior chamber cells and flare were more prominent in anterior uveitis than in other anatomical varieties. A proteinaceous flare was seen in the anterior chamber of only one patient with no uveitis, but appeared in over 90% of those with

anterior or generalized uveitis and in over half of those with posterior uveitis.

The relationship between the type of uveitis and the variety of keratic precipitates (KP) is well described in the literature.⁶¹ The patterns of KP in patients with generalized uveitis resembled those with anterior uveitis more than those with the posterior form. No patient without uveitis had KP. Mutton fat KP, supposed to be more common in "granulomatous" uveitis, were seen in about the same percentage of all patients with uveitis. Fine KP were described, as expected, in larger numbers among those with anterior uveitis (Table II).

Nonmicrobiological aqueous studies

Neither the darkfield wet preparation nor the anticomplement test revealed trep-

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TABLE II
COMPARISON OF 118 PATIENTS
ACCORDING TO ANATOMICAL VARIETY OF UVEITIS

	Anterior Uveitis	Posterior Uveitis	Generalized Uveitis	No Uveitis
Number of Patients	60 (69%)	11 (13%)	16 (18%)	31
Number of A.C. Taps	68	11	21	34
Age of Patients	31 (51%) Under 45 yrs.	4 (36%) Under 45 yrs.	12 (75%) Under 45 yrs.	21 (67%) Over 60 yrs.
Time Lapse from onset of Uveitis to A.C. Tap	12 (18%) Less than 72 hours	10 (90%) Over One Week	11 (52%) Over One Month	NA
Recurrences (six or more)	24 (35%)	0	6 (29%)	NA
History of				
Arthritis	10 (17%)	0	2 (12%)	3 (10%)
Sarcoid	4 (7%)	0	1 (6%)	0 (0%)
Diabetes	4 (7%)	1 (9%)	1 (6%)	6 (19%)
Syphilis	4 (7%)	3 (27%)	5 (31%)	4 (13%)
Active Uveitis	66 (97%)	8 (73%)	21 (100%)	NA
Biomicroscopy				
A.C. Cells	60 (88%)	6 (54%)	19 (91%)	0
A.C. Flare	64 (94%)	6 (54%)	20 (95%)	1 (3%)
Mutton Fat KP	15 (21%)	3 (27%)	5 (24%)	0
Fine KP	37 (55%)	2 (18%)	12 (57%)	0
Leishman's Stain of Aqueous				
Lymphocytes	51%	33%	39%	3%
Monocytes	14%	0	0	0
Mononuclear	41%	44%	61%	13%
Neutrophils	29%	0	0	6%
Macrophages	8%	0	11%	3%
Eosinophils	5%	0	0	3%
Basket Cells	14%	0	11%	0
WBC Bridging	21%	11%	44%	0
CBC (64 Patients)				
WBC (leukocytosis)	5/29 (17%)	3/6 (50%)	2/9 (22%)	1/22 (5%)
Hgb (reduced)	12/31 (39%)	0/6 (0%)	0/9 (0%)	5/23 (22%)
Eosinophilia	0	0	0	0
E.S.R. (elevated)	5/8 (62%)	2/4 (50%)	5/6 (83%)	1/2 (50%)
ANF abnormal	29/54 (54%)	1/7 (14%)	7/19 (37%)	9/17 (53%)

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onemes in the aqueous humor of most serologically positive persons. The darkfield was positive for spirochetes on 3 of 113 occasions (3%), and the anticomplement test on 4 of 53 occasions (7%).

While remaining aware of the possibility of aqueous *T. pallidum* in sero-negative patients,²³ the spirochetes seen in a fourth patient were suspect since no other tests suggested syphilis. Examples similar to those described in the literature as "spiral forms" were also noted.⁶⁵ When seen attached to white blood cells, they were thought to represent stromal strands.

Darkfield examination of the aqueous also revealed "bacteria or CWDF" in nine patients. From four of these, classical organisms were later recovered. Perhaps the organisms seen on the other five wet preparations were bacterial variants or organisms which could not be cultivated in our media.

Since over half of the 41 patients with some evidence of syphilis showed two or more positive indicators of this disease, the anticomplement test was expected to be more "positive". In this fluorescent staining technique for antigen-antibody complexes, the antigen (*Treponema pallidum*), when present in the aqueous and if coated with antibody and complement, reacts with fluorescein labeled antibody versus complement, causing the spirochete to fluoresce. The spirochete may have its cell wall damaged by a variety of forces (antibiotics, lysozyme, serum). Even though the classical spirochetal morphology might be considerably altered by such forces, it was hoped that the anticomplement test would be useful in detecting fragments of the cell wall of spirochetal CWDF.

Actually, the test is not specific for Treponemes. Spiral organisms seen to fluoresce in serologically negative persons may represent aberrant forms of nontreponemal species such as *Leptospira* or *Vibrio*. White blood cells with complement on the cell

surface may also take the stain and mimic spirochetes.

Table II shows aqueous cytology as evaluated with Leishman's stain in 120 of the 134 anterior chamber taps. White blood cells in the aqueous were tabulated as to their incidence of identification and their frequency as the predominant cell. When the red cell is the predominant or only cell in the aqueous, it probably reflects a traumatic anterior chamber tap, especially if the red cells look healthy. In one aqueous specimen, damaged or mottled RBC and basket cells were seen. The aqueous of this same patient harbored bacterial CWDF which did not revert, and nonreverting "cocci" were isolated from the circulating blood.

It can be impossible to differentiate lymphocytes from monocytes in the aqueous, and often we were forced to call unidentifiable cells "mononuclear" (Figure 6B).^{*} This is because the hypertonicity or the increased viscosity of the aqueous in uveitis compresses the cytoplasm so compactly around the nucleus that morphology becomes indistinguishable. Thus, the terms lymphocyte, monocyte and "mononuclear" were all used. Whether the presence of cells in the mononuclear series indicated the presence of bacterial CWDF (as McKay⁶⁶ found in swine), or whether they are an index of a hypersensitivity phenomenon is unknown. The lymphocyte⁶⁷ is known to be associated with an allergic response and is, in fact, the basis of a test for hypersensitivity states.⁶⁸

Of the cells in the mononuclear series, (lymphocytes, monocytes and "mononuclear" cells), lymphocytes are the most commonly identified aqueous cells in anterior uveitis. Rebeck⁶⁹ has shown a developmental progression in the mononuclear series from the small lymphocyte to the large mononuclear cell (or hypertrophying lymphocyte).

* Figure 6 is one of a series of special color plates that will appear in Part II in the Spring, 1978 issue of the Journal.

phocyte), and finally to the macrophage. This same inflammatory mononuclear progression has also been noted in the skin windows of uveitis patients.⁷⁰ In one third or more of the patients with each type of uveitis, various cells of the mononuclear series were the predominant cell. When the monocyte could be identified, it was always seen in anterior uveitis, never in other forms of uveitis. Hogan⁷¹ also found monocytes in the aqueous in anterior uveitis.

Neutrophils (Figure 6B) were found in almost one third of patients with anterior uveitis, but never in patients with posterior or with generalized uveitis. The neutrophil was the predominant cell in the aqueous of only seven of 120 anterior chamber taps, and in each instance the patient had anterior uveitis.

A macrophage by Downey's classification is a white blood cell which demonstrated phagocytosis. By these criteria, macrophages were seen in eight aqueous specimens. In only one specimen was the macrophage the predominant cell. In aqueous specimens from two patients with anterior uveitis, and from one with Eale's disease and another with heterochromia, macrophages had ingested a blue-black or purplish material thought to be uveal pigment. Similar phagocytosis of pigment by WBC has previously been described⁷⁰ in patients with uveitis when uveal pigment was used as an antigen or irritant under a Rebeck skin window.

In addition to the pigmented materials, macrophages in four aqueous specimens had phagocytosed materials interpreted to be "cocci". Bacterial CWDF which would not revert were recovered from three of the four cultures. In the fourth culture, classical organisms were isolated on one medium, but the nutritional requirements of this transitional form were incompletely understood, and it could not be maintained in cultures sufficiently long for speciation.

If the area of the cytoplasm exceeds that of the nucleus (a reverse ratio to the WBC), a cell is called an epithelial cell by Downey's classification. Epithelial cells were only rarely seen in the aqueous and may have arisen from the anterior chamber tap procedure.

Although there is an extensive literature devoted to the allergic or hypersensitive nature of the uveitis disease state, it is peculiar that the eosinophil plays almost no role within the aqueous response. The role of eosinophils can be impressive, however, in the skin windows of patients with uveitis when uveal pigment is used as the inflammatory stimulus.⁷⁰ Hogan states⁷¹ that in chronic iridocyclitis, "we have not yet identified an eosinophil" from the aqueous. In this study, eosinophils were noted only in aqueous specimens from patients with sarcoid, from two diabetics without uveitis, and from one patient with anterior uveitis. There might be reason to suspect eosinophils in one specimen since they characterize sarcoid.*

Basket cells (Figure 6C) are white cells which have been wasted in the inflammatory process. They appear as vacuolated, moth-eaten, depleted, "tired" cells. A battle has been waged in which they have expended everything. Although this cell is generally associated with active defense against microbial invasion, it can also indicate cellular

* Humphrey and White state: "Eosinophils in large numbers invade tissues in which an antigen-antibody has taken place. They appear to be attracted by some product of the antigen-antibody reaction. . . . The active agent has not been identified but is probably not histamine. The eosinophils of rodents are very actively phagocytic, and ingest cellular debris, mast cell granules, etc., but it is not certain whether this is true of eosinophils from other species, nor is it known what function eosinophils serve in these reactions. Nevertheless, the association of eosinophils with the occurrence of antigen-antibody reaction is so close that the presence of the one should always suggest the presence of the other."⁷²

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abuse from a variety of causes, such as aging, mechanical damage, or antibody bombardment. Work by Zieve, et al,¹⁰⁰ has been confirmed recently by Steigbigel, et al,¹⁰¹ indicating that the vacuolization of the neutrophil, at least in the circulating bloodstream, is an indication of septicemia. Our work with aqueous would tend to support this, since we found such vacuolated basket cells more frequently in the presence of microorganisms. Basket cells were identified in the aqueous specimens of 15 patients. None were seen in patients with posterior uveal involvement or without uveitis. They were identified in nine aqueous specimens from five patients with anterior uveitis. Bacterial CWDF were cultured from the aqueous of four of these five patients on one or more media; classical *Streptococcus fecalis* also grew from one. Basket cells were also seen in six other aqueous specimens from patients with generalized uveitis, and five of these specimens revealed cell wall defective bacterial forms on one or more media. Since basket cells were never seen in patients without uveitis, or with posterior uveitis, their presence may be significant.

Only once were plasma cells found in the aqueous of a patient with anterior uveitis. This patient had Eale's disease, and after 114 days of inflammation, a feeble plasma cell infiltration showed in the aqueous. Perhaps the absence of plasma cells in this study indicated a differential inflammatory response. Pavan-Langston⁷³ found a plasma response which began immediately following the induction of uveitis in rabbits with *Mycoplasma pulmonis* and lasted several weeks. At no time in this study were mycoplasma isolated. If there were plasma cells in the aqueous, perhaps mycoplasma would have been isolated as well. In one specimen, pleomorphic cells were noted by Leishman stain of the aqueous. This specimen was from any eye removed for choroidal melanoma subsequent to the anterior chamber tap.

In inflammation,⁹³ when injury to the endothelium⁷⁴ of small vessels occurs, capill-

ary permeability increases. The protein content of extravascular fluids increases (up to 6% or 7%), and there is increased stickiness of white blood cells. The resultant aggregation of white blood cells is similar to that seen in uveitis when protein produces aqueous flare, white blood cells aggregate, and KP are seen with the slit lamp.⁶¹ The clumping, chaining, aggregation or stringing together of white blood cells of the mononuclear series we call "bridging" (Figure 6B). Intracellular clumping or bridging must occur before white blood cells can effectively phagocytose. Cells which migrate to the inflammatory site first aggregate and then phagocytose.⁷⁵ Bridging in infection⁷⁶ is thought to be a response to pyogenic liposaccharides, and may be dependent on pH. Bridging, then, is a indication of an intact and effective defense mechanism. Tables II and III show our findings of bridging of WBC. The presence of bridging may be a tipoff to more effective host phagocytic mechanisms and thus help explain reduced numbers of microorganisms (Table III). Bridging can also be completely unrelated to host defense mechanisms and can be seen in rabbits under stress, hunger, fatigue, etc.⁷⁷ Under such circumstances it has been called "leukergy".

The sensitivity of the serological tests for syphilis increased among patients in this study from the VDRL (12% positive) to the RPCF (18% positive) and the Kolmer (20% positive) to the most sensitive FTA-absorption (36% positive). The total percentages of patients with a reactive serology were higher than the average number (18%) of patients reactive to these tests at the Henry Ford Hospital. The importance of the FTA absorption test is reemphasized. Assuming that it does not indicate false positives,⁷⁸ the test was three times as effective an indicator of syphilis as the VDRL, and even more frequently so than the aqueous darkfield (3% positive) and the anticomplement test (7% positive). These tests emphasize the variation which existed between the patients in this study without uveitis and any group of healthy controls. In 61% of the patients

TABLE III
A COMPARISON OF 70 PATIENTS WITH
ANTERIOR OR GENERALIZED UVEITIS

	32 Patients With Bacterial CWDF in Aqueous	38 Patients With No Bacterial CWDF in Aqueous
Number of A.C. Taps	39	44
Age (Patients under 60)	29 (91%)	20 (48%)
A.C. Tap within two days of onset of uveitis	5 (13%)	1 (2%)
History of:		
Arthritis	6 (19%)	5 (13%)
Sarcoid	4 (12%)	1 (2%)
First attack	15 (47%)	19 (50%)
Biomicroscopy		
A.C. Cells (3+ or 4+)	11 (28%)	8 (18%)
A.C. Flare (3+ or 4+)	11 (28%)	9 (20%)
K.P. Mutton Fat	10 (26%)	7 (16%)
Crenated	1 (3%)	8 (18%)
Leishman's Stain		
Aqueous WBC		
Neutrophils (Incidence)	12 (33%)	4 (9%)
Neutrophils (as predominant cell)	5 (14%)	2 (5%)
Basket Cells	3 (8%)	6 (16%)
WBC Bridging	6 (28%)	15 (40%)
Microbiological Findings		
Classical Bacteria in Aqueous	3 (8%)	0 (0%)
Acceptable Microbiological Studies on Blood	15	14
Bacterial CWDF in Blood	5/15 (33%)	0 (0%)
Classical Bacteria in Blood	2/15 (13%)	3/13 (23%)

NOTE: Does not include six A.C. taps which had microbiological contamination of specimens; nonaqueous specimens, or specimens from patients with posterior uveitis or no uveitis.

without uveitis there was a positive FTA-absorption test; the only other group as positive were patients with generalized uveitis.

Spirochetes can exist⁷⁹ in the spinal fluid without evidence of nervous tissue disease and can be present in the aqueous without obvious ocular disease⁸⁰ and with no serological indications. Christman⁸¹ found that in three of twelve patients the serology

was negative despite intraocular treponemes. *T. pallidum* can exist as a CWDF⁸², and it may exist as such when it persists *in vivo*.

Patients with anterior uveitis tend to be anemic. This may suggest a weakening of normal defense mechanisms, especially since abnormalities in the shape, size and hemoglobin content of the red blood cells

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were also commonest among patients with anterior uveitis.

None of 64 patients who had a c.b.c. showed eosinophilia. One might wonder why this cell is so rarely seen in the circulating blood, even when an episode seems a hypersensitive inflammatory response.

Patients with generalized uveitis were found to have the highest erythrocyte sedimentation rates. An alteration in basic defense mechanisms may allow these most severe episodes to develop.

The antinuclear factor (ANF) test was performed on most patients, but yielded little of interest. Although the number of abnormal ANF tests was high compared to the general populace, with 46 of 97 (47%) showing abnormal ANF, the ANF changes were non-specific. Interestingly, every patient with concomitant diabetes and syphilis had a positive ANF.

Some elevation of antibodies to bacterial antigens was noted, with an elevation of IGA levels in those patients with uveitis. IGA is thought to be partially responsible for the destruction of microbes in the aqueous. Elevation of IGM and IGG were less frequent but did occur. An increase in IGM commonly occurred concurrently with an abnormally high IGG. Such a change in an antibody response is consistent with a significant antigenic stimulus. The immune assays tend to support a theory of infection in relation to uveitis etiology.*

Our aqueous cytological and immunological findings tend to support a thesis statement that would incriminate organisms directly rather than hypothesize organismal

hypersensitivity as the primary etiological mechanism.

Analysis of all patients with anterior and generalized uveitis included a variety of patients with numerous additional variables. To eliminate some of these variables and further purify the groups of patients compared, several subanalyses were performed. In the first subanalysis, results from all diabetics (because of their known higher incidence of sepsis), allluetics (because organisms isolated might be assumed to be spirochetes), all laboratory accidents, and all nonocular specimens were eliminated from consideration. Then, by their clinical histories alone, the remaining patients were grouped into those patients expected to have bacterial cell wall defective forms in the aqueous and those not expected to have bacterial cell wall defective forms in the aqueous.

There were 42 patients expected to have aqueous bacterial CWDF because they had active interior uveitis, generalized uveitis, postoperative uveitis, sarcoid uveitis, or uveitis following perforating trauma. Twenty-four of these (57%) showed aqueous bacterial CWDF.

Among the group of 22 patients not expected to have bacterial CWDF, only four (17%) did. This was a group which had active herpetic uveitis, inactive anterior uveitis, posterior uveitis, presumed toxoplasmic uveitis, or uveitis in multiple sclerosis.

With the patients grouped in this way, it appears that these enigmatic bacterial forms may be of some clinical significance.

The second subanalysis of a selected group of patients is documented in Table III. Since patients with generalized uveitis are simply patients with more severe anterior uveitis, they were grouped together. The 32 patients in whom cell wall defective bacterial forms were recovered were then compared with 38 patients who had none of

* A later publication will present results of serial immunologic surveys on some of these patients, including serum immunoglobulin studies and antibody titers to various common bacteria and viruses.⁶³

these forms (Table III). Again, aqueous bacterial CWDF were found more frequently among younger patients (91% under 60 years old). And, again, CWDF were found more frequently among those who had early taps (within two days of onset of the attack). This might indicate that aqueous CWDF are present early in the attack, but disappear later. Perhaps, as humoral defense mechanisms become more effective, bacterial CWDF seek an intracellular uveal^{1,32,84} hiding place. The cell wall defective bacterial forms are known to have the capability of such intracellular survival; in one patient bacterial CWDF were not found in the aqueous, but were recovered from a snippet of iris removed during a surgical procedure.

No significant difference could be found in the incidence of associated nonocular infections between either group, although some earlier authors have suggested that such history is an important parameter. Eventually, it will probably be shown that such a history is significant.

An association between some forms of arthritis and bacterial CWDF has already been noted.⁴⁶ Table III shows that both a history of arthritis and a history of a tissue diagnosis of sarcoid occurred more frequently among patients with aqueous bacterial CWDF than among those without.

There is evidence to suggest that CWDF are an intracellular guest during periods of inactivity. Even if bacterial CWDF do play a role in uveitis, they might not be recovered from the aqueous if the disease were inactive. All patients in this subanalysis had active uveitis.

If bacterial CWDF do occur in the aqueous, a parameter related to uveitis might be the number of previous attacks. One pathogenic theory⁸⁴ proposes that bacterial CWDF flip in and out of a classical bacterial form and that this phasing in and out of pathogenicity explains recurrences. This theory would be buttressed by finding that bacterial CWDF were recovered more fre-

quently from the aqueous of patients with many recurrences of anterior uveitis than from patients with only one episode, since the latter group would include cases involving one-time-only etiologic factors. However, there was only very weak evidence that these enigmatic forms were recovered less frequently from patients having their first attack of anterior uveitis.

The presence of bacterial CWDF in active anterior or generalized uveitis may signal a more severe inflammatory process since both the cellular reaction and the proteinaceous flare, seen biomicroscopically, are more severe in the anterior chambers of those with aqueous bacterial CWDF (Table III). There may also be a relationship between the type of keratic precipitates (KP) and the presence of aqueous bacterial CWDF. Mutton fat KP were more frequently associated with their presence, and crenated KP were most frequently present if bacterial CWDF were not recovered. Since crenated KP are old KP, this tends to confirm the observation that if bacterial CWDF were present, they occurred during the early phase of a recurrence but were absent later.

Comparative results of the Leishman's stain of aqueous white blood cells were related to the presence of bacterial CWDF. Although McKay⁶⁶ found a mononuclear response at the site of swine lung tissue infected with the CWDF of *H. parainfluenza* and a neutrophilic response to classical organisms of that species, this study did not show that a mononuclear response was related to aqueous bacterial CWDF. In fact, better correlation existed between neutrophilic leukocytes and the presence of aqueous bacterial CWDF. The presence of neutrophils in the aqueous seems significant since they were seen in the aqueous of 33% of patients with bacterial CWDF in the aqueous, but in only 9% of those with no bacterial CWDF in the aqueous. In addition to a higher frequency of occurrence, neutrophils were also the predominant cell more frequently when bacterial CWDF were present

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(14%) than when they were not (5%). This neutrophilic aqueous response suggests that when bacterial CWDF are present, the host defense mechanism considers them significant.

The presence of CWDF in the aqueous was not associated with a peripheral leukocytosis, with a peripheral lymphocytosis or with a neutrophilia, nor did there seem to be any relationship between the presence or absence of aqueous cell wall defective forms in abnormalities of the shape, size or hemoglobin content of RBC's, the platelet count, or the erythrocyte and sedimentation rate.

The second part of Table III is an analysis of microbiological findings related to the presence of bacterial CWDF in the aqueous of patients with anterior and generalized uveitis.

It is interesting that bacterial CWDF were never found in the blood of patients without CWDF in the aqueous (none of 14), but were found in the blood of five of 15 patients from whom they were isolated from the aqueous. This suggests that bacterial cell wall defective forms, when present in the eye, are probably not limited to that organ.

There were classical bacteria present in the blood of about equal numbers of those patients with bacterial cell wall defective forms present in the aqueous and those with no bacterial cell wall defective forms present in the aqueous.

The occurrence of these generally anaerobic CWDF in the human microflora is relatively easy to establish. The significance of their presence in the eye or in other clinical specimens is not so easily assessed.

The third subanalysis is recorded in Table IV. It details parameters which may be related to the presence of bacterial cell wall defective forms in the aqueous of a group of patients with active anterior uveitis alone, and with no other known etiological influ-

ence. Thirty-one patients, on whom 34 anterior chamber taps were performed, were grouped for this subanalysis. These aqueous specimens were, then, from patients with "selected" or "pure" anterior uveitis. Again, those patients with aqueous bacterial CWDF were compared with those patients without.

The average age of patients with aqueous bacterial CWDF was lower than those without, and anterior chamber taps were performed earlier when aqueous bacterial CWDF were recovered.

Although the focus of infection theory⁸⁵ is generally discredited, there are cases in which the surgical extirpation of an infected organ abruptly stops a long series of recurrences. This puzzling fact is especially, though not exclusively, true of anterior uveitis. Some light may have been cast on this clinical peculiarity since six of the "selected" anterior uveitis patients who had bacterial CWDF in the aqueous also had a history of "associated infections", and five of them had a history of several or multiple foci. Only three (20%) of those without aqueous CWDF had a history of "associated infections", and none had multiple foci of infection. The evidence suggests that patients who have multiple foci of infection are more prone to episodes of anterior uveitis from which CWDF can be recovered. The "focus of infection" theory may not be completely dead.

The presence of aqueous and blood CWDF seemed to produce a leukocytic response in the peripheral blood smear. The findings suggest that patients who display a neutrophilic response in either the blood or the aqueous have a higher chance of positive microbiological studies.

The slit-lamp examination may also be correlated with the presence of aqueous CWDF. It may seem paradoxical that CWDF were found in the aqueous of all five selected patients who had "mutton fat" KP. In addition, crenated or old KP were seen twice as

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TABLE IV
COMPARISON OF FINDINGS IN PATIENTS WITH
"PURE" ANTERIOR UVEITIS

	16 Patients with Bacterial CWDF in Aqueous	15 Patients with No Bacterial CWDF in Aqueous
Number of A. C. Taps	18	16
Age under 45 years	9 (57%)	7 (46%)
A.C. Tap less than 72 hours from onset of uveitis	5 (28%)	9 (6%)
History of Associated Infections	6 (38%)	3 (20%)
History of Multiple Foci of Infections	5/16 (31%)	0
Biomicroscopy Mutton Fat KP Crenated KP	5/18 (28%) 4/18 (22%)	0 2 (11%)
Leishman's Stain of Aqueous Cells Neutrophils Basket Cells	8/16 (50%) 3/16 (19%)	3/12 (25%) 1/12 (8%)
Classical Bacteria in Aqueous	1	1
Bacterial CWDF in Blood	2/8 (25%)	0/5 (0%)
Peripheral Leukocytosis Peripheral Neutrophilia	3/12 (25%) 3/10 (30%)	0/4 (0%) 0/4 (0%)

NOTE: Excludes any known Etiology, Diabetics, Generalized Uveitis, Herpetic Uveitis, Microbiological Laboratory Accidents, No Uveitis, Posterior Uveitis, Sarcoid Uveitis, Syphilitics.

often on the corneal endothelium of the patients without bacterial CWDF. These findings suggest that CWDF, when present, are seen with more acute inflammation.

In this "pure" anterior uveitis grouping, neutrophils were present in the aqueous of over twice as many patients of the group with CWDF. They were also the predominant aqueous cell in 25% of these patients,

but never predominant if bacterial CWDF were not present. Once again, the neutrophil seemed to indicate that the presence of bacterial CWDF had some clinical significance.

Basket cells, indicators of host response to microbial invasion, were found more frequently in those with bacterial CWDF than in those without.¹⁰⁰ There appeared to be no

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difference in these subgroups in isolations of classical bacteria from the aqueous. One classical organism was recovered from each subgroup.

This subanalysis seemed to indicate some relationship between the presence of CWDF

in the aqueous and in the blood. Among the patients with bacterial CWDF in the aqueous, eight also had blood cultures and two (25%) had CWDF in the blood. In the group with no aqueous CWDF, five patients had blood cultures, but none was positive for CWDF.*

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*Studies on Uveitis, Part II, Observations with Case Reports, will appear in the Spring, 1978 issue of the Journal.

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

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