ENDOGENOUS OCULAR NOCARDIOSIS: A CLINICAL AND EXPERIMENTAL STUDY*

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Diseases desperate grown
By desperate appliance are reliev'd,
Or not at all.

Hamlet

INTRODUCTION

SHAKESPEARE PREDICTED A PRESENT-DAY MEDICAL DILEMMA. WITH THE INCREASing complexity and sophistication of modern medical science, a greater variety of drugs and procedures has evolved for the treatment of a wide range of diseases. With these medical advances, however, has come an increase in iatrogenic diseases. Ophthalmologists have witnessed postsurgical endophthalmitis caused by intraocular lenses, sympathetic ophthalmia following mechanical vitrectomy, steroid-induced glaucoma and cataracts, chloroquine retinopathy, cardiovascular diseases caused by antiglaucoma medications, radiation-induced sarcomas following treatment for retinoblastoma, and opportunistic infections of the eye associated with the use of potent immunosuppressive medications. These conditions represent the "trade-off" of the benefits from advanced medical technology.

Recent reports have described immunosuppressed or immunocompromised patients with ocular infections caused by opportunistic organisms not usually associated with disease in otherwise healthy patients. These infections include, among others, diseases caused by *Mucor, Cryptococcus,* cytomegalovirus, *Candida, Listeria, Aspergillus, Pneumocystis,* and *Nocardia.*

Nocardiosis with ocular involvement is not frequently encountered. Two previously unreported cases are introduced (case 1 was previously reported),[1] along with an animal model in which experimental endogenous nocardiosis was produced in the rabbit eye.

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BACKGROUND

NOCARDIA ASTEROIDES

Nocardiosis is typically caused by *Nocardia asteroides*. The organism was named for the starlike appearance of the colonies on agar plate (Fig 1) and for Edmond Isadore Etienne Nocard,[2] a French bacteriologist and veterinary pathologist born in 1850 in Provins, Seine-et-Marne. A protégé of Koch and Pasteur, Nocard, in 1888, became the director of the Alfort veterinary school, where his research in bovine peripneumonia, glanders, tuberculosis, rabies, hoof-and-mouth disease, sheep-pox, and other diseases[3] led to his isolating a filamentous organism from bovine farcy. This was, in 1889, named *Nocardia farcinica* by Trevisan.

Bovine farcy is now known to be caused by an unusual strain of *Mycobacterium* resembling *Nocardia*. The descriptions and cultures of the organism originally isolated by Nocard are inadequate to determine

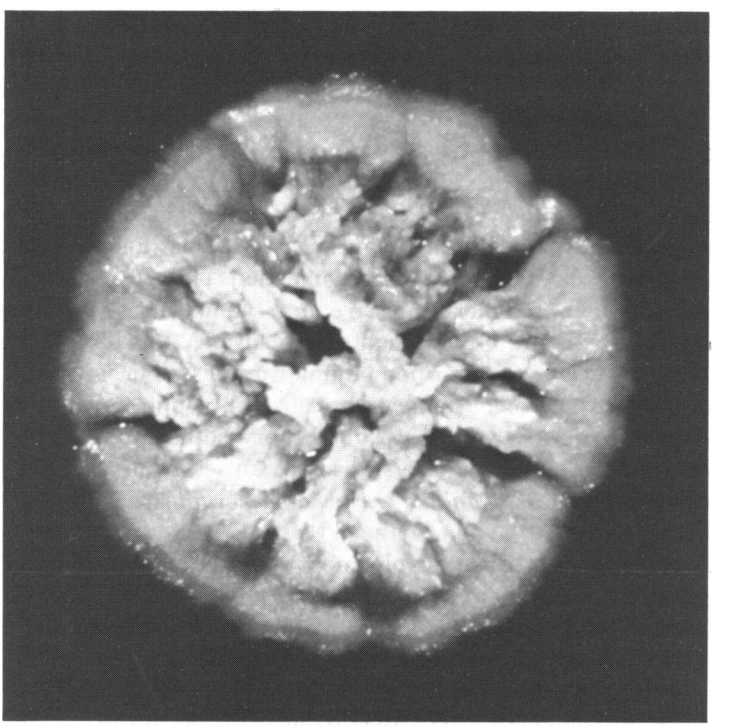

FIGURE 1
Typical starlike colony morphologic structure of *N asteroides* GUH-2 (BHI, 14 days, room temperature).

whether it was actually *Mycobacterium farcinogenes* or what is now classified as *N asteroides*. The latter was finally identified in 1896 by Blanchard from an organism Eppinger called *Cladothrix asteroides* after he isolated it in 1891 from the brain abscess of a patient with systemic nocardiosis.[4]

Nocardia asteroides, once thought to be a fungus, is classified in the bacterial family Nocardiaceae, which includes those aerobic actinomycetes having a complex cell wall that contains mesodiaminopimelic acid, arabinose, and galactose. The organism is soil-borne, reproducing by fragmentation of its hyphae into bacillary and coccoid elements, and is distinguished by a propensity for filamentous growth with true branching. It is a natural soil saprophyte and is often found in decaying organic matter such as wet hay or straw. *Nocardia asteroides* reduces nitrates to nitrites and does not hydrolize casein, tryosine, or xanthine. Esculin, allantoin, benzidine, and urea are hydrolyzed. Acid is produced from adonitol, arbutin, dextrin, D-fructose, D-glucose, and occasionally mannose and rhamnose.[5]

The organisms are gram-positive and often show an intermittent or beaded staining pattern, especially when grown in vivo. They are inconsistently acid-fast using the modified Kinyoun stain with 1% sulfuric acid as a decolorizing agent, and they stain black with the methenamine silver stain. In culture the organisms are not fastidious but tend to grow slowly. In pure culture the colonies often grow out after only 48 hours of incubation, but in mixed culture or in primary isolation from clinical material the colonies can take as long as 2 to 4 weeks to appear. The colonies can appear to be either smooth and moist, waxy and hard, or rough with a velvety surface caused by rudimentary aerial mycelium. They may have a heaped, waxy, or powdery starlike appearance and can be white, tan, buff, yellow, pink, red, orange, brown, or purple. The colonies will grow on virtually any bacterial, fungal, or mycobacterial media that lacks antibiotics and will grow within a wide temperature range. Growth of *N asteroides* is facilitated by 10% carbon dioxide.[6-13]

SYSTEMIC NOCARDIOSIS

Nocardiosis is a localized or disseminated infection that usually invades the body through the respiratory tract,[14] although the skin, mouth, intestinal tract, or eye can be penetrated with the introduction of the organism. When the lung is involved, the clinical picture can resemble a bronchitis or pneumonia similar to that seen with tuberculosis or other bacterial or fungal infections. Nocardial organisms can also cause mycetoma (also known as maduromycosis or Madura foot), a chronic, deep

subcutaneous tissue and bone infection, usually of the lower extremity.[15] *Nocardia asteroides* can be recovered from normal patients[16] as either an upper respiratory tract[17] or skin[18,19] saprophyte. Nocardiosis has a worldwide distribution and occurs in all races and ages. It is several times more common in men (aged 20 to 40) than in women and is reported much less frequently in young children and elderly adults than other age groups.

Between 20% and 50% of the cases of nocardiosis are in otherwise healthy patients,* but it is much more common in debilitated, immunocompromised, or immunosuppressed patients.[20-26] Patients who have an underlying condition such as systemic lupus erythematosus, chronic granulomatous disease, malignancy, alveolar proteinosis, organ transplantation, and/or corticosteroid therapy appear to have an increased risk for nocardiosis.[27-30] A follow-up study of heart transplant patients has shown a 10% incidence of nocardiosis.[29]

Nocardiosis usually appears as a pneumonia with a cough and low-grade fever. Malaise, weight loss, and night sweats may develop. The radiologic appearance is that of a lobar or segmental infiltrate that has developed rapidly. The disease becomes disseminated to the central nervous system (CNS) in 25% to 40% of the cases.[31] Ocular involvement has been reported in 3% of the cases of systemic nocardiosis[25]; however, few patients with nocardiosis have an eye examination. Thus, the true incidence of ocular involvement in nocardiosis cannot be estimated accurately. The diagnosis can be made by blood culture, sputum culture, or direct aspiration of material. Skin tests and serologic tests are unreliable. Specimens of sputum, pleural fluid, tracheostomy secretions, transtracheal aspirates, bronchial washings and brushings, and transtracheal biopsy specimens should be stained and cultured. A percutaneous lung biopsy or open lung biopsy may be necessary. If skin abscesses are present, these should be examined and cultured.

Most strains of *N asteroides* are sensitive to sulfonamides, the treatment of choice.[32] Six to 10 gm of sulfadiazine or sulfisoxazole are given daily. Treatment must be prolonged (a minimum of 6 weeks in immunologically intact patients and 1 year in immunosuppressed patients). It is recommended that immunosuppressive medications be reduced in patients infected with nocardiosis. Prognosis in nocardiosis depends upon the extent of the disease, the acuteness of the condition, and the patient's underlying immunologic status. Patients who are immunologically intact

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*Bing Crosby developed a pulmonary abscess (simulating carcinoma) as a result of aspirating a piece of lettuce contaminated with *Nocardia*.

and otherwise healthy have only a 15% mortality from nocardiosis. Non-immunosuppressed patients with an underlying disease have a mortality of approximately 20% from the nocardiosis, while patients who are taking immunosuppressive medications and have nocardiosis have a mortality of 80% to 100%.[33] Surviving nocardiosis depends upon the accuracy and aggressiveness of the antimicrobial treatment and upon the discontinuation or marked reduction of steroids and/or other immunosuppressive medications. Early diagnosis is essential for successful therapy.[33]

PREVIOUS MODELS OF SYSTEMIC NOCARDIOSIS

Numerous studies[13,34,35] have been performed using a variety of experimental animals to investigate nocardial pathogenicity. Rabbits and guinea pigs have been infected, but most of the experimental work has involved mice.[36] The results of the previous studies have been inconsistent and contradictory because of differences in the types of animals used, in the various strains of *Nocardia*, in the routes of administration of the organism, and in the use or nonuse of an adjuvant. In all models, however, a lethal disease could be produced.

NOCARDIAL PATHOGENESIS

Nocardia asteroides is a facultative, intracellular parasite that can persist and grow within macrophages. No exotoxins have ever been described for nocardiae.[36] *Nocardia asteroides* usually does not multiply quickly enough within host tissues to produce a fulminant, overwhelming infection; nocardiosis is usually a chronic, progressive disease.[36]

The nocardial cell wall is important in its pathogenicity, and it is composed of a variety of complex lipids, peptides, and polysaccharides.[37] Chemical differences in the cell walls of differing strains and at differing stages of growth may explain differences in pathogenicity. Iron seems to be a necessary factor for the intracellular growth of most nocardial organisms.[36,38]

HOST DEFENSE AGAINST NOCARDIOSIS

The exact mechanisms of defense against nocardial infections are not completely understood. Defense against infectious diseases, in general, represents a combination of innate immunity and specific acquired immunity. Innate immunity consists initially of those factors that prevent entry of the organism into the body. These include the skin, with its normal flora, fatty acids, lactic acid, sebaceous secretions, and low pH, and the mucous membranes with their mucus, cilia, tears, lysozyme, and

normal flora. Innate immunity also consists of those factors that initially counteract an invading organism. These include humoral factors such as C-reactive protein, α-1 antitripsin, α-2 macroglobulin, fibrinogen, acute-phase proteins, and interferon. Phagocytosis is accomplished by the polymorphonuclear neutrophil, the larger macrophages, and the other cells of the reticuloendothelial system. Phagocytosis occurs when the microbial organism is engulfed by the macrophage; a phagosome is then formed that fuses with the lysosomal granule to form a phagolysosome. Within the phagolysosome, the ingested organisms are destroyed by a number of potent hydrolytic and proteolytic enzymes. An extremely important part of the innate immunity system is complement, the cascading system able to destroy microorganisms.[39,40]

Specific acquired immunity consists of the interaction of humoral immunity and cell-mediated immunity. Many diseases caused by opportunistic organisms are thought to represent a malfunction of cell-mediated immunity. Both humoral antibodies and delayed-type hypersensitivity to nocardial antigens have been found in persons with systemic nocardiosis.[36] Histopathologic studies have shown that neutrophils may also be involved in the defense against *N asteroides*.[41] These findings would indicate that specific and nonspecific immunologic factors would all function to combat the *N asteroides*. When attempts have been made to examine the role of each specific component of the immune system, however, the exact nature of that component has become less clear.

Nonspecific Immunity

Filice et al[42] studied the effect of human neutrophils and macrophages on *N asteroides* and showed that these cells are unable to kill significant numbers of this organism in vitro. In the same assay system, however, the neutrophils and monocytes were able to kill *Listeria monocytogenes* and *Staphylococcus aureus*. It was determined that the failure to kill *N asteroides* was not associated with an absence of an oxidative metabolic burst, the absence of which does occur in chronic granulomatous disease. Nocardiosis has been recorded in patients with chronic granulomatous disease in which there is a defect in the oxidative killing mechanisms in polymorphonuclear leukocytes, monocytes, and macrophages,[43,44] and it has been speculated that *N asteroides* is resistant to the metabolites of the oxidative burst. Some organisms such as *Brucella abortus* and *Toxoplasma gondii* are able to survive intracellularly by actually inhibiting the oxidative metabolic burst, while other organisms can survive the metabolic burst by converting superoxide to oxygen and hydrogen peroxide. In *N asteroides*, however, the concentration of superoxide dismutase has been

found to be comparable to levels found in many other organisms that are susceptible to killing by neutrophils and monocytes.[45]

Specific Acquired Immunity

Humoral Immunity.—There is evidence concerning the lack of importance of humoral immunity in nocardial infections. Beaman et al[46] studied experimental nocardial infections in B-lymphocyte-deficient mice. These mice have B-lymphocytes that are defective in their ability to interact with macrophages that present bacterial antigens to the lymphocytes, and because of this, reduced levels of serum IgM and IgG_3 antibodies that would act against *Nocardia* are found. These mice, however, did not show increased sensitivity to lethal infection from *N asteroides* in spite of measurable differences in antibody levels. Cell-mediated immunity remains intact in these B-lymphocyte-deficient mice. However, the B-lymphocyte-deficient mice were 1000 times more susceptible to *Salmonella typhimurium* infection than were normal mice. These findings suggest that resistance to nocardiosis (in distinction to salmonellosis) is independent of humoral immunity. An in vitro study was published by Davis-Scibienski and Beaman.[47] They found that immune serum enhanced slightly the phagocytosis of *N asteroides* by activated macrophages; however, the antibody did not result in increased macrophage resistance to nocardial infection. Both rabbit and human macrophages have been shown to have receptors for the Fc component of IgG.[48-50] These receptors increase in number and affinity after macrophage activation[51] and have been shown to be important for particle attachment and ingestion.[48,50,52] This would imply that humoral immune factors merely assist the cell-mediated immune system, which can function adequately in the absence of specific antibody.

Cell-Mediated Immunity.—Beaman and co-workers,[53] in 1978, showed that athymic mice were significantly more susceptible than their heterozygous litter mates or normal mice to challenge with *N asteroides*, suggesting that T- (thymus-derived) lymphocytes are important in protection against nocardial infections.

Filice and colleagues[54] studied the effects of activated macrophages on *N asteroides* and found that when cellular monolayers of nonspecifically activated macrophages were used, the monolayers were still intact, and the growth of *N asteroides* was markedly inhibited. In contrast, with control macrophages, extensive arborization of nocardial filaments was observed, and it appeared as if nocardial filaments had extended through neighboring macrophages. The organisms that grew within control macrophages were not acid-fast, whereas those that grew within the activated

macrophages were strongly acid-fast. Enhanced resistance of *Nocardia* to decolorization with acid-alcohol reflects altered lipid composition of the cell envelope and may be related to enhanced ability to evade destruction by host defenses. Marked protection was also observed in mice that had nonspecifically activated macrophages as a result of infection with another facultative intracellular organism such as *Listeria*.[55]

Phagocytosis of most organisms by macrophages is normally followed by rapid fusion of macrophage lysosomes with phagosomes.[56] Avoidance of exposure or resistance to the hydrolic environment following release of lysosomal enzymes may contribute to the survival of some intracellular parasites,[57] and it has been shown by Davis-Scibienski and Beaman[58] that this same phenomenon exists with *N asteroides*. They have also shown a direct correlation between the virulence of several different strains of *N asteroides* and their resistance to ingestion and killing by macrophages. When these investigators labeled phagocytic lysozymes by pinocytosis of horseradish peroxidase or by the use of lysosomal acid phosphatase cytochemistry and then examined these cells by electron microscopy, the more virulent strains of *N asteroides* showed a markedly reduced frequency of lysosome-phagosome fusion. Studies using acridine orange fluorescent microscopy likewise showed a marked reduction in lysosome-phagosome fusion when the virulent *N asteroides* GUH-2 was phagocytized by the macrophage. They also found that formalin-killed virulent GUH-2 organisms were as effective as viable organisms, suggesting that nocardial inhibition of phagosome-lysosome fusion probably arises from a component of the cell surface. It has been shown that anionic sulfolipids isolated from the cell walls of virulent *Mycobacterium tuberculosis* inhibit lysosome-phagosome fusion in mouse macrophages.[59] Similar anionic compounds in virulent *N asteroides* may be responsible for the inhibition of fusion. Thus, the virulent GUH-2 strain of *N asteroides* not only resists phagocytosis and inhibits phagosome-lysosome fusion if it is ingested, but it also appears to be resistant to the phagolysosomal environment when fusion does occur.

Beaman et al[60] studied the effects of *N asteroides* on germ-free mice and found that they respond in a manner significantly different from that of conventionally raised mice after intravenous injection of *N asteroides*. These germ-free mice are much more sensitive to the organism, since they have had no prior contact with a resident microflora and therefore, presumably, no activated macrophages. The normal flora of conventionally housed (normal) mice appear to activate continuously, but nonspecifically, the host defense capabilities against nocardial infection. Macrophages from germ-free mice have been shown to have decreased en-

zymes, decreased ability to spread on glass, and decreased ability to phagocytize particles by way of C_3b complement receptors.[61] The authors feel that their observations further establish the necessary role of the activated macrophage and the development of cell-mediated immunity in host resistance to infection by *N asteroides*.[60]

The experimental data cited previously suggest that all components of the immune system interact within the host to provide effective protection against *Nocardia*. A deficiency, therefore, in any factor of the immune system may compromise the host with respect to nocardiosis.

MECHANISMS OF ACTION OF IMMUNOSUPPRESSIVE MEDICATIONS

Steroids

Corticosteroids have many effects on the immune system. They increase adenyl cyclase, cyclic adenosine 3′:5′monophosphate (AMP), enhance prostaglandin E, and potentiate the effects of β-adrenergic catecholamines.[62-65] They diminish the release of the vasoactive amines (histamine, heparin, and serotonin) from mast cells and basophils.[62] Steroids constrict blood vessels[66] and diminish vascular permeability.[67] They reduce neutrophil chemotaxis[68] and induce monocytopenia,[69] eosinopenia, and lymphopenia. They inhibit lymphocyte proliferation, function, and circulation,[70,71] and they depress the proliferative response to mitogenic stimulation by antigenic stimuli. Corticosteroids reduce macrophage metabolism, impede macrophage circulation, and interfere with antigen retention on the macrophage surface.[72] Steroids reduce the movement of antigen-antibody complexes across basement membranes.[67]

T-lymphocytes are more sensitive to steroids than are B-lymphocytes,[73] but the suppression of cell-mediated immunity by steroids is mainly due to macrophage inhibition rather than to T-cell suppression.[74] Steroids stabilize lysosomal granules and inhibit the release of lysosomal enzymes, thereby preventing the destruction of phagocytized intracellular organisms.[75]

More recent work has shown subcellular effects of steroids in many aspects of the inflammatory response, including a decrease in the production of T-cell growth factor[76] and a decrease in the number of Fc receptors on phagocytic cells.[77,78] In addition, steroids have been shown to have profound effects on lymphocytes by inhibiting T-cell function, especially the function of helper T cells.[79]

Cyclophosphamide

Cyclophosphamide is an alkylating agent similar to the nitrogen mustards, which are among the most potent chemical immunodepressants

available. Cyclophosphamide itself is not an active immunosuppressant, but it is metabolized to form alkylating compounds.[80] Cyclophosphamide shows a great selectivity for lymphoid tissue, wherein it produces a rapid and profound decrease in cellularity with a resultant lymphopenia. Cyclophosphamide damages deoxyribonucleic acid (DNA) and restricts the cell's ability to complete mitosis. In nonstimulated lymphocytes, the DNA can repair itself, but in rapidly proliferating, stimulated lymphocytes, the repair is more difficult.[80] Because of this, cyclophosphamide is most effective as an immunosuppressant when it is given during the most sensitive time period: from 48 hours before to 48 hours after antigenic challenge.[81] Its most profound immunologic response is on antibody formation, and this effect is attributable to its antiproliferative activity. It also affects delayed hypersensitivity,[82,83] antigen-induced lymphocyte transformation,[84] and migration-inhibition-factor production.[85] In some situations, however, the cyclophosphamide can actually increase delayed hypersensitivity, possibly by its greater effect on suppressor T cells than on effector T cells.[86] Cyclophosphamide has been reported to prolong the survival of organ or skin allografts in certain species.[87-89] This is due to a depression of cell-mediated cytotoxicity.[90] Starzl et al[91] have found cyclophosphamide, antilymphocyte globulin, and steroids to be the best immunosuppressive combination in hepatic and renal transplant patients. Cyclophosphamide induces a tolerance to soluble as well as to particulate antigens.[92,93] Cyclophosphamide eliminates stimulated, proliferating clones of cells when given near the time of antigen injection and also affects macrophages by reducing antigen-trapping in lymph nodes.[94]

The prime effect of cyclophosphamide, however, is on the B-lymphocyte.[95,96] Large injections of cyclophosphamide deplete B cells and increase the relative percentage of T-lymphocytes. Since it preferentially affects proliferating cells, it would be most toxic to short-lived lymphocytes, which are primarily B cells.[80] Thus, cyclophosphamide exerts a profound influence on the immune system.

EFFECT OF IMMUNOSUPPRESSION ON NOCARDIOSIS

Experimental

Animal models of experimental nocardiosis have been produced by several investigators,[34,97,98] most commonly using mice. Beaman and Maslan[97] showed that cyclophosphamide treatment of mice 72 hours prior to infection dramatically increased host susceptibility to nocardial infection. In addition, cyclophosphamide greatly enhanced the ability of the nocardial strain to grow within the various organs and significantly altered normal

host clearance from these organs. These authors found that cyclophosphamide altered host response to nocardial infection more profoundly than did comparable doses of cortisone, prednisolone, or azathioprine.

Mishra et al[98] showed that the susceptibility of white mice to nocardiosis was unmistakably enhanced by cortisone. They determined that test strains of *Nocardia* caused a more extensive disease and a greater and more rapid mortality in steroid-treated mice than in normal mice. The LD_{50} (lethal dose for 50% survival) values of nocardial organisms were seven to eight times lower when steroid immunosuppression was used.

Clinical

Cross and Binford[99] reported that 20.4% of the human cases of nocardiosis had been immunosuppressed with steroids, thus giving a clinical correlation to the above-mentioned laboratory investigations.

HUMAN OCULAR NOCARDIOSIS

Human ophthalmic involvement from *N asteroides* occurs by two separate mechanisms, exogenous and endogenous. Most of the reported cases concern the exogenous form of the disease.[100-115]

Exogenous

In the exogenous form, the ocular nocardiosis develops as a result of a superficial infection with or without trauma and with or without intraocular extension.

Endogenous

In the endogenous form, the ocular nocardiosis can occur in an immunologically normal, immunosuppressed, or immunocompromised patient. In this form of the disease, the organisms reach the eye through the bloodstream. Endogenous nocardiosis has been reported previously on ten occasions.[1,116-124] Table I is a summary of the clinical and histopathologic findings in these cases.

PREVIOUS OCULAR MODELS

There have been two previous attempts to create an animal model of experimental ocular nocardiosis, but neither succeeded in producing the endogenous form of the disease, ie, in reproducing the fundus appearance seen in humans. McCarthy and associates[100] implanted the organism into the right anterior chambers and left superior fornices of six rabbits. The eyes were then treated with a daily topical prednisolone-neomycin ointment preparation. Four of the six animals died of nocardiosis. These

TABLE I: CLINICAL AND HISTOPATHOLOGIC SUMMARIES OF PREVIOUSLY REPORTED PATIENTS WITH ENDOGENOUS OCULAR NOCARDIOSIS*

REFERENCE	PATIENT AGE/SEX	PREDISPOSING FACTORS	SOURCE OF POSITIVE CULTURE(S)	OCULAR SYMPTOMS	INVOLVED EYE(S)	CLINICAL OCULAR FINDINGS	TREATMENT (DOSE)	OCULAR HISTOPATHOLOGIC FINDINGS	OUTCOME
116	50/M	Chronic renal failure; renal transplant; immunosuppressed with azathioprine, corticosteroids, antilymphocyte globulin; radiation therapy; antibiotic therapy (kanamycin sulfate, tetracycline)	Submandibular mass; perinephric abscess, vitreous and posterior bulbar space	Pain, blurred vision	Left	Subretinal mass; cloudy vitreous	Sulfisoxazole, 6 gm/d; cycloserine 750 mg/d; enucleation	Nonspecific necrotizing chorioretinitis	Alive and well 31 mo past onset of ocular symptoms
117	67/M	Gunshot wound; penicillin therapy; "antibiotic therapy"	Lung; cutaneous ulcer	Not stated	Left	Peripapillary choroidal retinal inflammation superior to macula	Penicillin; sulfadiazine; cycloserine	Acute necrosis and inflammation of choroid and retina; organisms seen in retina, choroid, and subretinal space	Death

TABLE I: CLINICAL AND HISTOPATHOLOGIC SUMMARIES OF PREVIOUSLY REPORTED PATIENTS WITH ENDOGENOUS OCULAR NOCARDIOSIS* (CONTINUED)

REFERENCE	PATIENT AGE/SEX	PREDISPOSING FACTORS	SOURCE OF POSITIVE CULTURE(S)	OCULAR SYMPTOMS	INVOLVED EYE(S)	CLINICAL OCULAR FINDINGS	TREATMENT (DOSE)	OCULAR HISTOPATHOLOGIC FINDINGS	OUTCOME
117	56/M	None	Evisceration material	Not stated	Both	OD: chorioretinal lesion OS: chorioretinitis, proptosis, lateral orbital mass	Iodides; enucleation of OS	Lymphocytic and polymorphonuclear leukocyte infiltration around organisms seen in Bruch's membrane, retina, sclera, and cornea	Not stated
118	46/M	Gallbladder disease; systemic corticosteroids	Vitreous cavity	Decreased vision	Left	Anterior uveitis, macular lesion with nonrhegmatogenous retinal detachment	Enucleation; triple sulfa	Chronic iritis, cyclitis, and choroiditis; extensive necrotic subretinal abscess; organisms seen in subretinal space	Alive and well

TABLE I: CLINICAL AND HISTOPATHOLOGIC SUMMARIES OF PREVIOUSLY REPORTED PATIENTS WITH ENDOGENOUS OCULAR NOCARDIOSIS* (CONTINUED)

REFERENCE	PATIENT AGE/SEX	PREDISPOSING FACTORS	SOURCE OF POSITIVE CULTURE(S)	OCULAR SYMPTOMS	INVOLVED EYE(S)	CLINICAL OCULAR FINDINGS	TREATMENT (DOSE)	OCULAR HISTOPATHOLOGIC FINDINGS	OUTCOME
119	20/M	Explosion injury to extremities; penicillin therapy; tetracycline; cephalothin; kanamycin sulfate; chloramphenicol; gentamycin	Blood; abscesses of knee, penis, and subcutaneous sites; vitreous cavity	Foreign body sensation and decreased vision	Both	OD: conjunctival injection, exudative retinal detachment OS: multiple small retinal abscesses resembling "staphylococci colonies on blood agar"; after healing, left fundus showed "punched out" chorioretinal lesions in former areas of whitish exudate	Sulfadiazine; enucleation, OD	Hemorrhage; vitreous abscess with total retinal detachment; chronic iridocyclitis; organisms seen in vitreous abscess	Alive and well 7 mo past onset of ocular symptoms
120	24/F	Systemic lupus erythematosus; prednisolone therapy, 30-60 mg/d; cyclophosphamide; "broad spectrum" antibiotics	Sputum; subcutaneous abscesses	Not stated	Right	Acute uveitis with exophthalmos; periorbital edema and conjunctival injection	Triple sulfonamides 6 gm/d; streptomycin 500 mg/d; cotrimoxazole 6 tablets/d; cycloserine 750 mg/d	No ocular specimen	Alive and well

TABLE I: CLINICAL AND HISTOPATHOLOGIC SUMMARIES OF PREVIOUSLY REPORTED PATIENTS WITH ENDOGENOUS OCULAR NOCARDIOSIS* (CONTINUED)

REFERENCE	PATIENT AGE/SEX	PREDISPOSING FACTORS	SOURCE OF POSITIVE CULTURE(S)	OCULAR SYMPTOMS	INVOLVED EYE(S)	CLINICAL OCULAR FINDINGS	TREATMENT (DOSE)	OCULAR HISTOPATHOLOGIC FINDINGS	OUTCOME
121	77/F	Malignant lymphoma; radiation therapy; prednisone; cyclophosphamide, vincristine, antibiotics	Pulmonary and brain abscesses	Blind, OD	Right	OD: retinal exudates; later, severe vitreous haze; ptosis OS: sixth nerve palsy; bilateral facial palsy	None for *Nocardia*	Thick subretinal exudate; thickened, inflamed choroid; typical organisms seen	Patient died 6 wk after exudates were noted OD
122	38/M	Hypogammaglobulinemia; chronic aggressive hepatitis; prednisone 50 mg/d; isoniazid	Lung abscess and pleural effusion fluid; vitreous (from vitrectomy); subcutaneous abscess; blood	Pain and decreased vision, OS	Both	OD: anterior uveitis; vitritis; hemorrhage, exudate in posterior pole OS: hemorrhagic exudate in posterior pole	Sulfadiazine; trimethoprim-sulfamethoxazole; minocycline; erythromycin	OS: first eye involved had no organisms seen histopathologically OD: lymphocytic and polymorphonuclear infiltration in retina and choroid with numerous organisms seen	Patient died 6 mo after onset of ocular symptoms
123	60/M	Hodgkin's disease; prednisone 60 mg/d; antibiotics	Lung	Pain and redness, OD	Right	Hemorrhagic exudate, right fundus	None for *Nocardia*	Necrotic retina and choroid with typical organisms seen; lymphocytic and polymorphonuclear infiltration	Patient died several weeks after onset of ocular symptoms

TABLE I: CLINICAL AND HISTOPATHOLOGIC SUMMARIES OF PREVIOUSLY REPORTED PATIENTS WITH ENDOGENOUS OCULAR NOCARDIOSIS* (CONTINUED)

REFERENCE	PATIENT AGE/SEX	PREDISPOSING FACTORS	SOURCE OF POSITIVE CULTURE(S)	OCULAR SYMPTOMS	INVOLVED EYE(S)	CLINICAL OCULAR FINDINGS	TREATMENT (DOSE)	OCULAR HISTOPATHOLOGIC FINDINGS	OUTCOME
124	23/M	Systemic lupus erythematosus; renal transplant; azathioprine 150 mg/d; prednisone 40 mg/d	Bronchial washings	None	Right	Subretinal mass	Trimethoprim-sulfamethoxazole	Granulomatous suppuration with typical organisms seen	Alive and well 2 yr after ocular involvement

*Modified from Clarkson and Green.[142]

investigators produced a pathologic picture consisting of granuloma formation with microabscesses. They also produced interstitial keratitis, vascularization of the cornea, lymphocytic infiltration into the anterior chamber angle, edema of the iris and ciliary body, bladder cell formation at the equator of the lens, hyperemia of the choroid, subretinal exudates, scleritis and episcleritis, and a nocardial dacryoadenitis. They were able to produce lesions in 58% (7 of 12) of the infected eyes.

Newmark and co-workers[101] inoculated the corneas of 12 pigmented rabbits with a suspension of *N asteroides* filaments. They treated six rabbits with a normal saline solution, four times daily, and six rabbits with a topical 0.1% dexamethasone ophthalmic solution, four times daily, for 3 weeks. In the eyes treated with steroids, large granulomatous lesions with extension into the anterior chamber developed in two animals, while the saline-treated eyes showed small lesions that eventually healed by vascularization. No intraocular extension of the infectious process was produced.

Even though these experimental studies were done well, they failed to produce an animal model that accurately reflected the disease as seen in systemically immunosuppressed patients.

CASE REPORTS

CASE 1*

A 40-year-old white man was in good health until the onset of fatigue and arthralgias in 1967. Eventually, a diagnosis of Wegener's granulomatosis was made. The patient was treated with corticosteroids and azathioprine, which resulted in an initial improvement. In 1969, progressive deterioration of renal function was treated with chronic hemodialysis. In November 1971, the patient underwent a cadaveric renal transplant. Azathioprine, 2 to 3 mg/kg, and high doses of prednisone were given to prevent rejection of the renal transplant.

The transplanted kidney functioned well until February 1972, when the patient was hospitalized for evaluation of chills and fever. *Nocardia asteroides* was cultured from abscesses on the arm and back. Sulfadiazine, 8 gm/day, was administered for 1 week, after which sulfisoxazole, 16 gm/day, was given for the next 5 weeks. A generalized rash prompted the

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*This case was previously reported and was seen by the present author during the patient's hospitalization. Published with permission from the *American Journal of Ophthalmology* 1973; 76:568-573. Copyright by the Ophthalmic Publishing Company.

discontinuation of the sulfisoxazole. The skin abscesses resolved. On May 3, 1972, deterioration of the renal function prompted a biopsy, the results of which showed acute rejection. High-dose prednisone therapy was reinstituted. Daily febrile spikes were noted after 2 weeks, and blurred vision was noted 1 week later.

On May 24, visual acuity was 20/60 in the right eye (OD) and 20/25 in the left eye (OS). Four elevated white-yellow choroidal infiltrates with overlying retinal detachment and retinal hemorrhages (Fig 2) were noted OD. The left fundus was normal. Multiple nodules were present in both lungs on roentgenographic evaluation. Miliary abscesses, obtained by open lung biopsy, grew out *N asteroides*.

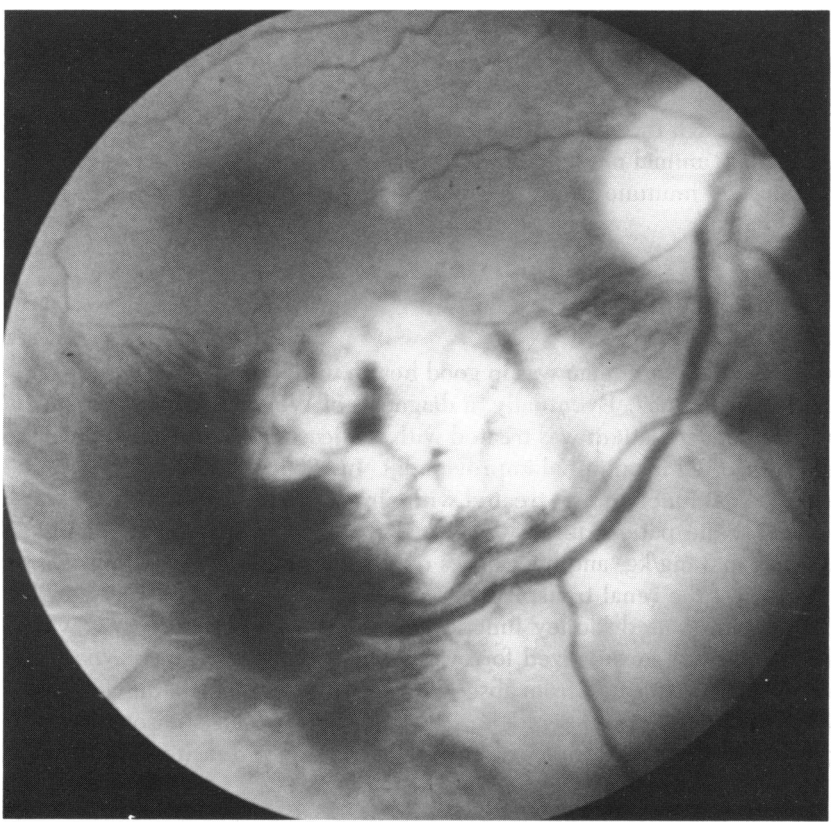


FIGURE 2
Case 1. Fundus of right eye showing elevated yellow-white choroidal infiltrate with overlying retinal hemorrhages.

Despite therapy with sulfisoxazole, ampicillin, and streptomycin, the patient's condition deteriorated. The choroidal lesions did not change before the patient died on June 11.

General Pathologic Findings

There was evidence of mild rejection in the transplanted kidney, but no organisms were cultured. *Nocardia asteroides* was identified only in the prostate gland and OD.

Pathologic Findings in the Eye

Four distinct chorioretinal infiltrates were noted OD. A marked infiltration by lymphocytes, plasma cells, and polymorphonuclear leukocytes was noted in the choroid. The Brown-Brenn stain demonstrated *N asteroides.*

CASE 2*

A 15-year-old white boy was seen in June 1974, complaining of the recent onset of decreased vision in each eye. His past medical history was complex and significant in many respects. At age 10, he was first noted to have anemia with dark urine. This improved spontaneously, but 1 year later, the anemia recurred and persisted. After 1 more year, a diagnosis of paroxysmal nocturnal hemoglobinuria was made and prednisone therapy was instituted. This was continued intermittently and was later increased to 60 mg daily because of an apparent increase in anemia and hemoglobinuria. The recurrences appeared to be related to upper respiratory tract infections, flu episodes, other infections, increased activity, and antibiotic treatment. The bone marrow at that time was hyperplastic.

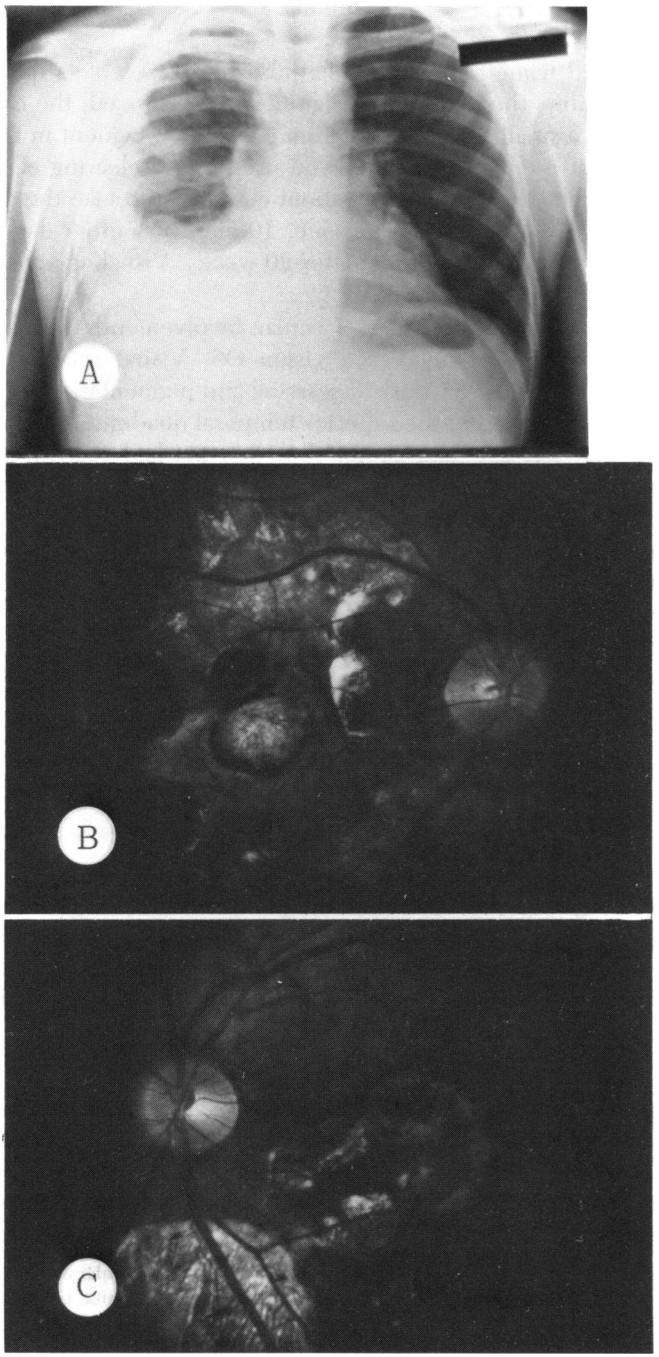
Lymphocyte antigen typing was performed on the patient and his family. A sister was found to be an excellent match, with incompatibility noted in only the Rh antigen series. At age 13½, the patient underwent a bone marrow transplant from his sister with an excellent hematologic result. Following that procedure, however, obstructive jaundice developed. A liver biopsy suggested an intracanalicular obstruction. His clinical findings included jaundice, progressive weight loss, pruritis, and acholic stools. The bilirubin level was markedly elevated. The jaundice was associated with markedly elevated alkaline phosphatase levels. These chemical findings and the liver biopsy specimen appearance were consistent with a drug cholestasis phenomenon. (The patient had been exposed to compazine therapy in the past.) In addition, a graft vs host reaction was diagnosed and treated with antilymphocyte globulin and methotrexate. Cholestyramine and vitamins A, D, and K were administered.

*This patient was seen while the author was working at the Mayo Clinic.

At age 15, the patient was hospitalized with a 1-month history of a nonproductive cough, low-grade fever, and right-sided chest pain that was exacerbated with coughing and deep breathing, all of which were unresponsive to erythromycin therapy. The cough produced dark yellow, non-foul-smelling sputum. The patient had shortness of breath and dyspnea on exertion. He was then given three injections of an unknown antibiotic over a 2-day period. On admission, his temperature was 37.6° C, the pulse rate was 100 beats per minute, and the blood pressure was 100/76 mm Hg. He was in poor nutritional state and was markedly jaundiced and slightly cushinoid. The most remarkable findings were limited to the chest and abdomen. Examination of the chest revealed decreased breath sounds over his right chest anteriorly and in the left lower lobe posteriorly. A few rales were heard on auscultation. No rubs or rhonchi were noted. A chest roentgenogram showed an opacification of the right base with a diffuse infiltrative process in the right upper and middle lobes and a large loculated pleural effusion on the right (Fig 3A). The abdomen was soft, and the liver was palpable. The sputum showed many yeast forms, many gram-negative cocci, gram-positive cocci, and a few gram-negative bacilli. Several days after admission, the patient complained of a black spot in front of his right eye and blurring of vision in the right eye. Visual acuity was 20/320 OD and 20/30 OS. Bilateral scleral icterus was noted. The pupils, extraocular muscles, and visual fields were normal. Dilated fundus examination showed bilateral round, yellowish, creamy-looking chorioretinal exudates with surrounding hemorrhages located in three areas: (1) the right macula, (2) superotemporal to the right macula, and (3) inferior to the left disc. A clinical diagnosis of *Nocardia* chorioretinitis was made on the basis of these ophthalmoscopic findings and their similarity to the appearance of the fundus of the patient in case 1.

A smear of a transtracheal aspirate showed acid-fast hyphal elements most compatible with *Nocardia*. Branching hyphae were also noted on direct smear and Gram's stains of the sputum. *Nocardia* organisms were cultured from the blood, from the sputum, and from a transtracheal aspirate of pulmonary secretions. Treatment with trimethoprim-sulfamethoxazole was begun, 125 mg orally twice daily. In addition to the antimicrobial therapy, a program of intense physiotherapy and postural drainage was instituted. The prednisone was tapered rapidly from 60 mg daily to 10 mg every other day. The hospital course was one of slow but progressive improvement. The chest pain gradually diminished, and facility of respiration increased. There was no evidence of CNS involvement from the nocardiosis.

The patient was discharged on a regimen of prednisone, 10 mg every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other day, and trimethoprim-sulfamethoxazole, two tablets daily orally. One month later, the patient was significantly improved; the cough was minimal with a small amount of sputum production evident in the morning. The chest roentgenogram showed progressive clearing of the infiltrate in the right lung base. The patient continued to take the trimethoprim-sulfamethoxazole and prednisone, 10 mg every other day. Antimicrobial therapy was discontinued after 20 weeks. Visual acuity stabilized at 20/200 OD and 20/30 OS.

Eight months after the onset of ocular involvement, the patient returned complaining of decreased vision OS. Visual acuity measured 20/400 OD and 20/60 OS. Marked scarring and pigmentation were noted in the right macula and in the superior temporal quadrant of the right eye (Fig 3B). The left eye had similar but less marked scarring two disc diameters below the disc, with two fingerlike projections from this lesion toward the fovea (Fig 3C). Initially, a diagnosis of recurrent nocardial chorioretinitis was made, but the diagnosis was later changed to left subretinal neovascularization with hemorrhage. Six weeks later, visual acuity was 20/200 in each eye. Three months later, visual acuity was 20/200 OD and 20/100 OS with persistent hemorrhage in the left macula. Four months later, visual acuity was 20/100 OD and 20/60 OS with healing of the lesions. Two years after initial eye symptoms, visual acuity measured 20/200 OD and 20/60 OS at distance, and 20/100 OD and 20/30 OS at near.

CASE 3*

A 59-year-old white man consulted a physician on Dec 12, 1979, complaining of leg aches, a dry throat, and pain, redness, and a foreign body sensation in the left eye. There was no history of ocular trauma. The patient related a history of childhood polio and a recent 9-kg weight loss. Three months earlier, the patient had spent several days shoveling moist,

*I did not examine the patient in case 3 but was consulted for the postmortem microscopic examination.

FIGURE 3

Case 2. A: Chest roentgenogram 3 days prior to onset of visual symptoms, showing opacification of right base with diffuse infiltrative process in right upper lobe and right middle lobe, and large loculated right pleural effusion. B: Fundus of right eye, 8 months after onset of visual symptoms. Marked scarring and pigmentation are seen in macular area. Visual acuity was 20/400. C: Fundus of left eye taken at same time as Fig 3B. Scarring, pigmentation, and subretinal neovascularization with hemorrhage are seen in macular area. Visual acuity was 20/60−. (Figs 3B and 3C are courtesy of the Mayo Clinic.)

decaying hay within the confines of his barn. A diagnosis of "mild viral syndrome" was made. Vitamin supplements were prescribed, and the patient returned 3 weeks later with a deterioration in the condition of his left eye. Examination at that time revealed a cloudy left cornea and a partially opaque anterior chamber. The patient was referred to an ophthalmologist, who made a diagnosis of "metastatic carcinoma to the left eye," prescribed steroid-antibiotic and mydriatic drops, and suggested a systemic workup. A chest roentgenogram, chemistry profile, and complete blood cell count were all within normal limits, with the exception of an elevated blood sugar level. The patient was admitted to a community hospital, with the chief complaints of weakness, weight loss, difficulty in walking, and pain in the left eye. A general physical examination was unremarkable. The intravenous pyelogram, barium enema, gallbladder roentgenogram, upper gastrointestinal roentgenography series, and liver and spleen scans were all within normal limits. The blood sugar levels were elevated, and the patient was given a 1400-calorie diabetic diet; chlorpropamide, 250 mg orally twice daily, was prescribed. An isotopic brain scan showed accumulation of radioactivity in the occipital lobes, which was believed to be compatible with either a cerebral infarct or tumor. An electroencephalogram was within normal limits. The patient was then transferred to another hospital because of a low-grade fever (0.7 to 1.3° C) and gradual, progressive mental deterioration. Numerous purpuric spots and a few excoriated papules were noted on dermatologic examination. Ophthalmologic examination demonstrated an opacified left cornea with exudate in the anterior chamber and severe conjunctival inflammation. The lungs were clear. Palpable inguinal and right axillary lymph nodes were noted, and the prostate gland was more than three times normal size. The neurologic examination showed decreased strength in the left leg. A chest roentgenogram showed left hilar prominence with minimal fine nodular changes.

One day after admission, decreased memory was noted. A CT scan showed a lesion with contrast enhancement and surrounding cerebral edema was demonstrated in the left occipital region and in several additional areas within the right occiput (Fig 4A and B). An additional lesion was noted in the right parasagittal area. The chest roentgenogram again showed slight hilar prominence (Fig 4C). An isotope brain scan showed abnormal uptake in the left posterior parietal area, deep and close to the midline, and an additional small area of abnormal uptake in the right frontal region. A gallium scan showed multiple abnormalities in the brain, an abnormal area of uptake in the left lung, and abnormalities in the left orbit and right mastoid areas. A carcinoembryonic antigen level was

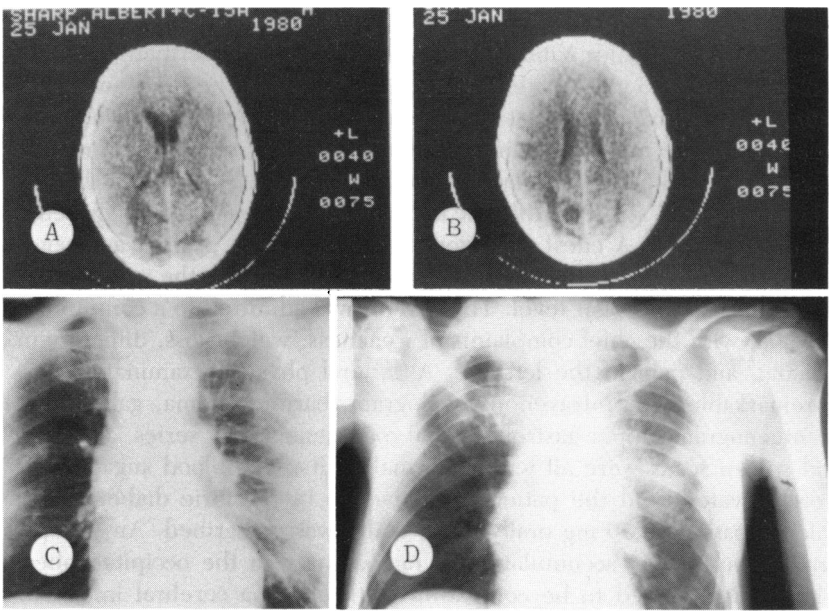

FIGURE 4
Case 3. A and B: Cranial CT scan showing lesion with contrast enhancement and surrounding cerebral edema in left occipital region and several additional areas within right occiput. C: Chest roentgenogram showing slight hilar prominence with linear perihilar infiltration into left thorax. D: Repeat chest roentgenogram 4 days later, showing left upper lobe infiltrate with frank bilateral hilar adenopathy.

reported as 2.6 ng/ml, which was within normal limits. The sedimentation rate was 49 mm/hour.

Four days after admission to the second hospital, the patient had an episode of hemoptysis. A repeat chest roentgenogram showed a left upper lobe infiltrate, possibly secondary to intrapulmonary blood, aspiration, or bacterial pneumonia with frank bilateral hilar adenopathy (Fig 4D). Intravenous penicillin was given. The following day, the patient had a severe episode of hemoptysis, developed labored respirations, and died.

General Pathologic Findings

Autopsy revealed two separate light-green masses in the left anterior chamber obscuring the left pupil. The left thoracic cavity contained 1250 ml of liquefied and clotted blood. Gross blood and blood clots were noted in the major bronchi, especially on the left side. The left lung weighed 930 gm. The lower lobe was firm, containing blood except for the most superficial peripheral part of the basal lobe. A 3.5-cm mass was present in

that area of the lung, and there was a gaping 2-cm hole within the mass (Fig 5A). A blood clot filled the bronchial tree. The entire upper lobe was consolidated with recent hemorrhage, indicating a bronchial communication. The surface of the kidneys showed multiple yellow-white areas ranging in size from 1 to 2 mm (Fig 5B). The prostate was mildly enlarged. The tail of the epididymis contained yellowish-white material from which cultures were taken. Coronal sections of the cerebral hemisphere showed multiple yellowish-green abscesses, ranging from 6 to 15 mm, present in the right and left frontal, parietal, and occipital areas (Fig 5C). Two abscesses were also present in the cerebellar hemisphere. The midbrain, pons, and cerebellum elsewhere showed no other abnormalities.

Microscopic examination of the right and left cerebral and right cerebellar hemispheres, the kidneys, and the lungs showed many gram-positive hyphae seen with the Brown-Brenn stain.

Fragments of tissue obtained from the eye, brain, epididymis, lung, and kidneys grew *N asteroides* on blood agar plates.

*Ocular Pathologic Findings**

The left eye measured 18 × 23 × 24 mm with a 1-mm segment of optic nerve. The cornea measured 9 × 11 mm. A 3-mm central corneal perforation was present. White cheesy material was present in the flat anterior chamber. The vitreous cavity was opaque.

An unhealed central corneal perforation with sharp wound margins was present (Fig 6A). The wound was filled with incarcerated iris and fibrovascular inflammatory tissue. A large nodule of acute and chronic inflammatory cells was present in the adjacent conjunctiva. The remaining cornea was edematous and filled with acute inflammatory cells. The iris was necrotic, its stroma replaced with acute inflammatory cells. The angle was occluded by inflammatory cells and peripheral anterior synechiae. The retina was totally detached by inflammatory cells and subretinal exudate. The retinal architecture was preserved anteriorly, but posteriorly it was disrupted by inflammation and hemorrhage (Fig 6B). The vitreous contained acute inflammatory cells (Fig 6C). Diffuse acute and chronic nongranulomatous inflammation was present in the choroid (Fig 6D). The Brown-Brenn stain revealed numerous branching filamentary organisms in the choroid and vitreous cavity, consistent with *Nocardia* (Fig 6E).

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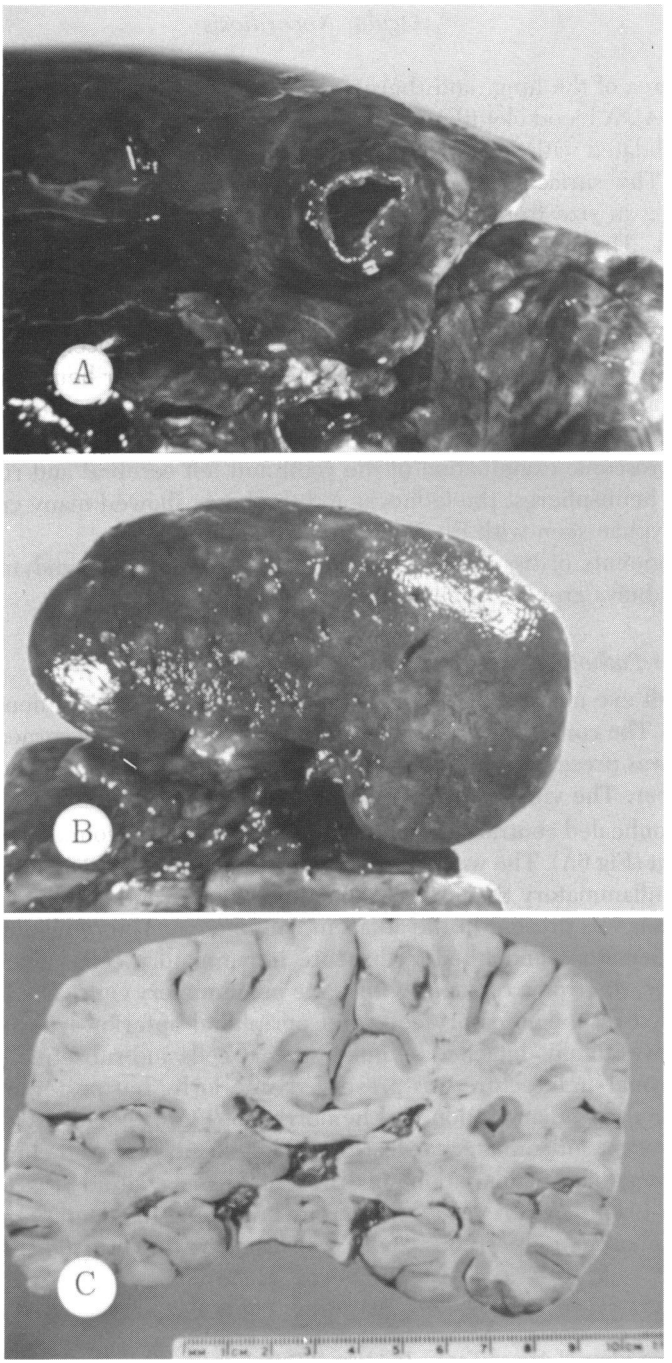
*Report courtesy of Armed Forces Institute of Pathology.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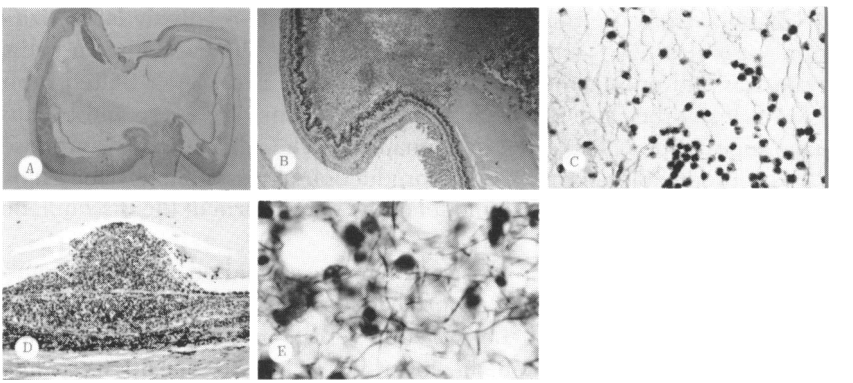


FIGURE 6

Case 3. A: Whole-mount photomicrograph of left eye showing massive thickening of choroid, an opaque vitreous, and flattening and dehiscence of anterior segment (hematoxylin and eosin). B: Same eye showing massive inflammation of choroid and retina (hematoxylin and eosin, ×30). C: Vitreous cavity of same eye showing acute inflammatory cells (hematoxylin and eosin, ×500, oil). D: Same eye showing choroidal abscess with overlying retinal detachment (Brown-Brenn, ×250). E: Same eye showing numerous gram-positive beaded branching nocardial organisms (Brown-Brenn, ×2500, oil).

EXPERIMENTAL STUDIES

INTRODUCTION

Laboratory animals have been used in several studies testing the relative pathogenicity of *Nocardia*. The problems in some of these studies were a lack of standardization of bacterial culture age and growth conditions and the use of a variety of animal hosts.[125] To date, animal models are generally not available for testing drug therapy for nocardiosis.[17,25]

In the present study, Dutch-Belted rabbits, immunosuppressed by treatment with steroids, with or without cyclophosphamide, were challenged with a standardized inoculum of 10^3 CFU (colony-forming units) of *N asteroides*, strain GUH-2. The introduction of the bacteria by way of the carotid artery produced infection with visible lesions in the retina. Observation of the retinal lesions verified the presence of disseminated disease in the living animal. Lesions were also found throughout the internal organs at autopsy. Intravenous injection produced a systemic disease with a much lower incidence of ophthalmic changes.

FIGURE 5

Case 3. A: Gaping 2-cm hole in left lung. B: Kidney showing multiple yellow-white surface nocardial abscesses, ranging in size from 1 to 2 mm. C: Coronal section of brain showing multiple yellowish-green abscesses ranging from 6 to 15 mm.

Rabbits were used in this study because of the relatively large size of the carotid arteries and the eyes, and because standard human retinal cameras and ophthalmoscopes could be used. The Dutch-Belted rabbit was selected because of the brown pigmentation of the eye and because the appearance is similar to the human eye. The rabbit model is proposed as a reproducible method for the production of disseminated nocardiosis as well as for production of a model of the ocular form of the disseminated disease.

Four different antimicrobial regimens were studied using this rabbit model. Previous experimental work has shown highly inconsistent results in vitro, inconsistent and contradictory results in vivo, and in general, a lack of correlation between the in vivo and in vitro sensitivity results. Variation in susceptibility of different strains of *Nocardia* was demonstrated by Bach et al,[126,127] who tested 45 antimicrobial agents and found minocycline to be consistently active in vitro while sulfa was inactive. Runyon[128] found streptomycin, aureomycin, penicillin, and sulfadiazine to be effective in vitro. In their in vivo studies, only the sulfadiazine decreased the mortality of mice infected with *Nocardia*. Penicillin produced paradoxical results: 60% of control mice and 100% of penicillin-treated mice died from the nocardial infection. With aureomycin, a 2-mg subcutaneous injection reduced mortality from 35% (control mortality) to 10%; however, increasing the dose of aureomycin afforded no protection. Only sulfadiazine was consistently effective both in vitro and in vivo.

Strauss and co-workers[129] noted the importance of animal studies in chemotherapeutic investigations: Certain *Nocardia* strains that were resistant to sulfadiazine in vitro were highly sensitive in vivo. They noted, in contrast to Runyon's findings,[128] that increasing the dose of aureomycin did increase the survival rate of the infected mice. Chloramphenicol was effective at a daily dosage of 25 mg/kg, but doubling of the dosage resulted in the death of all animals even though a daily dosage of 50 mg was well tolerated by uninfected animals. Strauss et al[129] proposed that it was a combination of the nocardial infection and the drug toxicity at this higher dosage that resulted in the death of the animals.

Chemotherapeutic studies in humans indicated that a number of antimicrobials may be effective in the treatment of nocardiosis: sulfonamides,[26] cycloserine,[130,131] erythromycin,[127] ampicillin (alone or in combination),[126,132] amikacin sulfate,[133] tetracycline and minocycline,[126,127] and sulfamethoxazole-trimethoprim combinations.[134,135]

Given the inconsistent and conflicting results of previous in vitro studies, it was difficult to select meaningful antimicrobial regimens and dosages for therapeutic trials with the experimental infection. The lack of a

good animal model for testing drug therapy for nocardiosis had been noted by Frazier et al.[17] The rabbit model for disseminated nocardiosis is proposed and used in the present study for the in vivo testing of a selected group of antimicrobial agents. A total of 211 rabbits were used for the entire experimental study.

RABBIT MODEL OF DISSEMINATED NOCARDIOSIS

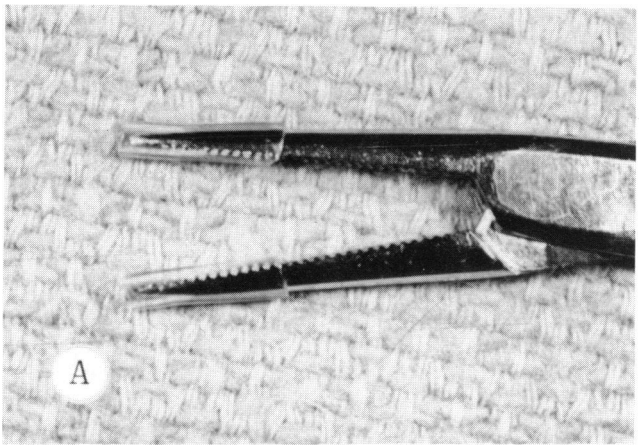
Fifty-five rabbits were used in a study to develop an animal model of disseminated nocardiosis.

Materials and Methods

Dutch-Belted rabbits, weighing from 1 to 2 kg, were kept in an approved animal facility with food and water ad libitum.

Nocardia asteroides, strain GUH-2, isolated from the kidneys of a fatal case of nocardiosis and maintained by transfers in the mouse kidney, was provided by Blaine L. Beaman, PhD, University of California, Davis. The culture was grown in Brain Heart Infusion Broth (BHI, Difco) for 72 hours at 34° C with mild agitation (150 rpm, New Brunswick Scientific Controlled Environment Incubator Shaker). This stock culture was frozen at −70° C in 15% vol/vol sterile glycerine. Cultures were handled in a method modified from Beaman and Maslan.[97] The stock culture was subcultured to fresh BHI and grown to log phase for 16 hours at 34° C. The culture was centrifuged at 100 g for 15 minutes, and the pellet was resuspended in 0.85% saline. Resuspended bacteria were pooled, diluted in BHI to give 10^4 CFU/ml, and made 15% vol/vol with sterile glycerine. Aliquots of 2 ml were dispensed to sterile tubes and frozen at −70° C. These cultures were thawed and diluted with saline to 10^3 CFU/ml just prior to use. Colony counts were repeated for each experiment to verify the inoculum. The count remained constant over a 24-month period after initial freezing.

Three days before the injection of the bacteria, rabbits were anesthetized with ketamine hydrochloride (40 mg/kg, intramuscularly [IM]; Vetalar, Parke-Davis). Rabbits were shaved in the neck area, and a hair cream (Surgex, Cooper Laboratories) was applied to remove the residual hair. The rabbits were pretreated with saline or with steroids with or without cyclophosphamide. Three days later, the rabbits were again anesthetized with ketamine hydrochloride and were given the *Nocardia* via the carotid artery or marginal ear vein. For the carotid injection, a fine hemostat with tips covered by plastic tubing was used to clamp the carotid artery. The plastic prevented damage to the artery (Fig 7A). A 30-gauge needle was used to puncture the common carotid artery distal to the clamp, and a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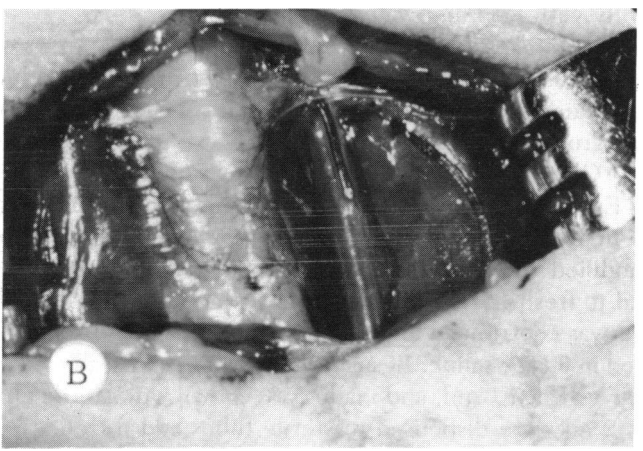
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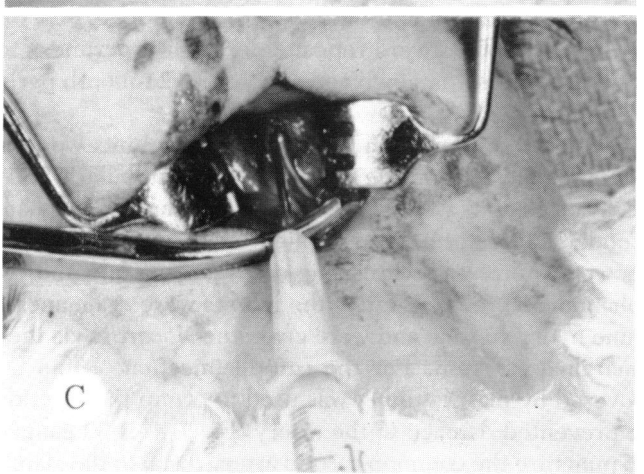
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1-ml inoculum of 10^3 CFU/ml of *Nocardia* was injected over 10 seconds (Fig 7B and C). After the injection, the needle was withdrawn, the skin incision was closed, and the animal was returned to its cage and given food and water ad libitum. For the marginal ear vein injection, the ear was shaved and the marginal ear vein was dilated by rubbing a xylene-soaked gauze pad over the ear. The vein was entered with a 26-gauge needle, and the injection was given over a 3- to 5-second period.

The internal organs and eyes of the rabbits that died or were killed by CO_2 inhalation were removed to 10% phosphate-buffered formalin. The tissues were embedded in paraffin and sections prepared. Sections were stained with HPS (hematoxylin, phyloxine, and safranine), the Brown-Brenn modification of Gram's stain, Gomori's methenamine-silver stain, and the Fite modification of the acid-fast stain. Microscopic sections were examined under oil immersion for evidence of nocardial infection. Microscopic sections were photographed with color slide film (Ektachrome 50, Eastman Kodak Company). Twelve uninfected rabbits were used as tissue controls: 6 were untreated; 2 were treated with 6-methylprednisolone, 30 mg/kg; 2 were treated with cyclophosphamide, 100 mg/kg; and 2 were treated with both 6-methylprednisolone and cyclophosphamide 3 days before they were killed.

The differences in rabbit mortality among the various groups of experimental treatments were analyzed by using the chi-square method, with the Yates' correction.

Results

Bacterial Inoculum.—Preliminary studies were carried out to determine the virulence of *N asteroides*, strain GUH-2, for Dutch-Belted rabbits. A disseminated disease without acute mortality was necessary to allow time for future studies of host-parasite interactions. Four rabbits were given injections of 10^6 or 10^7 CFU by carotid puncture, the approximate LD_{50} in mice.[97] All four animals died within 48 hours. *Nocardia* was cultured from the kidney, although neither gross nor histologic lesions were visible at that time. Other internal organs were not cultured.

Sham-Operated Controls.—Nonimmunosuppressed. As Table II indicates, four rabbits received a saline injection followed 3 days later by an intra-arterial injection of saline as a control for the surgical procedure, and all four animals survived the 28-day test period (group A, Table II).

FIGURE 7

A: Fine hemostat with tips covered with plastic tubing to prevent damage to carotid artery during clamping. B: Surgical isolation and identification of left common carotid artery of rabbit. C: Injection of left common carotid artery of rabbit using 30-gauge needle attached to 1-ml tuberculin syringe. Note clamp proximal to puncture site.

TABLE II: EFFECT OF PRETREATMENT IMMUNOSUPPRESSION ON RABBIT MORTALITY IN EXPERIMENTAL NOCARDIOSIS

GROUP	PRETREATMENT MEDICATION	DOSAGE	NO RABBITS	ROUTE OF BACTERIAL INJECTION*	10^3 CFU *N ASTEROIDES*	MORTALITY RATE (%)†
A	Saline	. . .	4	IA	–	0
B	6-Methylpredniso-lone	30 mg/kg	4	IA	–	0
C	Saline	. . .	7	IA	+	0
D	6-Methylpredniso-lone	30 mg/kg	16	IA	+	100
E	6-Methylpredniso-lone	30 mg/kg	4	IV	+	100

*IA = intra-arterial; IV = intravenous.
†At 28 days.

Immunosuppressed. Four rabbits were pretreated with 6-methylprednisolone, 30 mg/kg, and injected intra-arterially with saline as a control for the effect of the steroid and the surgical procedure. The four animals all survived the 28-day test period (group B, Table II).

Nocardial Injection.—Nonimmunosuppressed. Seven rabbits (group C, Tables II and III) injected intra-arterially with 10^3 CFU* of *N asteroides* GUH-2 were still alive at the end of the 28-day test period and were used as controls (Tables II and III). Four uninfected, nonimmunosuppressed, intact, actively growing rabbits also served as controls. The nonimmunosuppressed *Nocardia*-infected rabbits all appeared healthy and were gaining weight during the 28-day test period (Fig 8). They continued to gain weight until they were killed, 6 weeks to 4 months after bacterial injection. Gross pathologic findings of the internal organs of these infected rabbits revealed fine, healed lesions (Fig 9A and B).

Four additional nonimmunosuppressed rabbits received an intravenous (IV) injection of 10^3 CFU *N asteroides* GUH-2. Two rabbits were killed 3 days after injection and two rabbits were killed 10 days after injection. Histopathologic examination of the lungs and kidneys was performed on all four animals. No lesions were noted at 10 days. No renal lesions were noted at 3 days. The lungs of the two animals killed at 3 days showed a few scattered parenchymal lesions consisting of alveolar histiocytes and lymphocytes with rare polymorphonuclear leukocytes in a micronodular response. No necrosis was noted and no organisms were seen (Fig 9C and D).

*The 10^3 CFU figure was empirically chosen as the geometric mean between one *Nocardia* organism (10^0) and the lethal dose (10^6). Because of limited funds, supply of rabbits, cage space, and other practical details, intermediate levels were not tried.

TABLE III: EFFECT OF STEROID PRETREATMENT DOSE ON RABBIT MORTALITY FOLLOWING INTRAVASCULAR CHALLENGE WITH 10^3 CFU *NOCARDIA ASTEROIDES* GUH-2

GROUP	DOSE OF INTRAMUSCULAR 6-METHYLPREDNISOLONE (mg/kg)	NO RABBITS	ROUTE OF BACTERIAL CHALLENGE*	MORTALITY RATE (%)†
D	30.0	16	IA	100
F	20.0	6	IV	100
G	10.0	6	2 IV/4 IA	33
H	5.0	6	3 IV/3 IA	50
I	2.5	6	3 IV/3 IA	0
C	0.0	7	IA	0

*IA = intra-arterial; IV = intravenous.
†At 28 days.

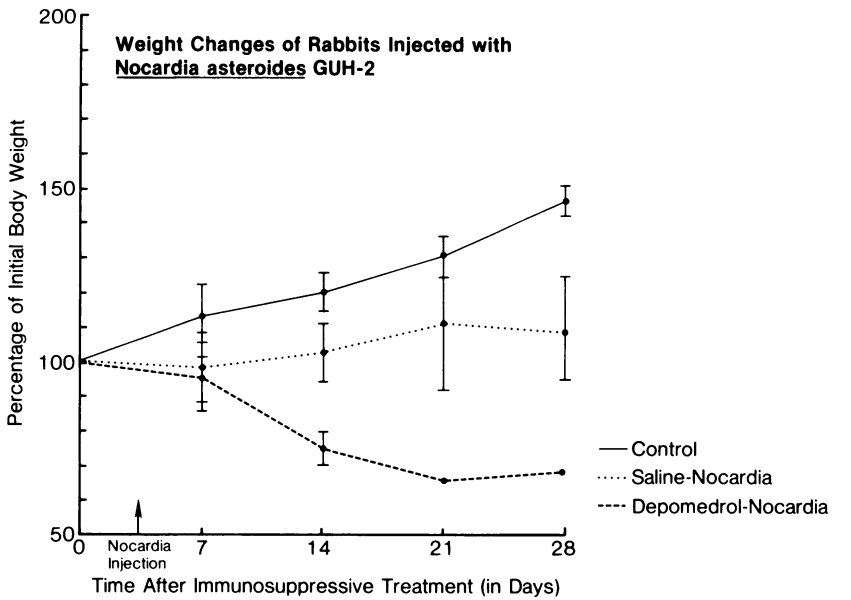


FIGURE 8
Percentage of body weight changes of rabbits as a function of time following immunosuppressive treatment with 6-methylprednisolone (Depo-Medrol) and infection with *N asteroides* GUH-2.

Immunosuppressed. Intra-arterial Injection. Sixteen rabbits (group D, Tables II and III) were pretreated with 30 mg/kg of IM 6-methylprednisolone (Depo-Medrol), 72 hours prior to the bacterial challenge. All 16 rabbits died between 8 and 21 days after the nocardial injection (Tables II and III). The combination of immunosuppression and the bacterial infection resulted in weight loss (Fig 8) and mortality in these animals. The results of gross pathologic examination of the internal organs revealed large, creamy abscesses. Lesions were found in the lungs (Fig 10A), ear, spleen, kidneys (Fig 10B), heart, pericardium, liver (Fig 10C), pleura, peritoneum, brain (Fig 10D), and bowel. Histologic examination showed an overwhelming infection associated with a marked degree of necrosis. Multiple necrotic abscesses were noted, especially in the kidneys and lungs. Of note was the very minimal cellular response and the marked number of nocardial filaments in the tissues (Fig 11). Nuclear debris was especially evident.

Intravenous Injection. To provide an animal model that could be duplicated more easily under routine laboratory conditions, four rabbits (group

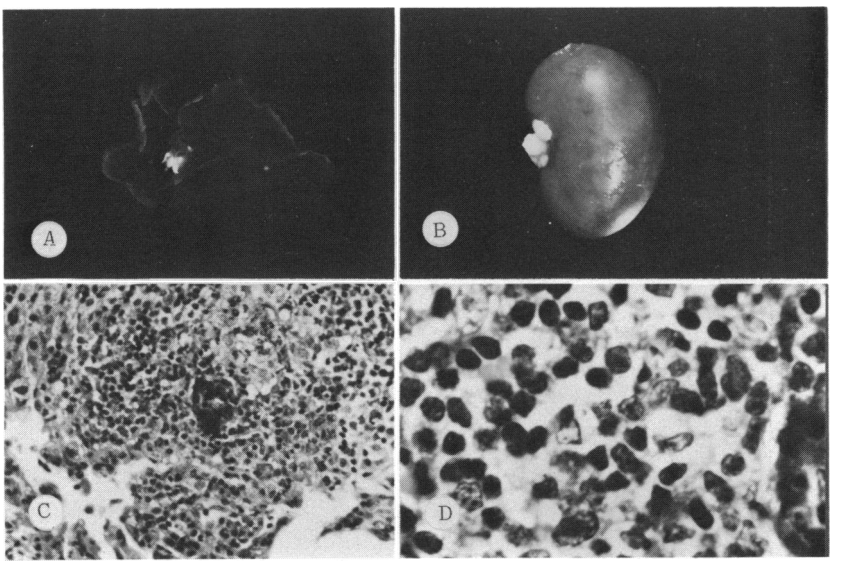


FIGURE 9

A: Typical fine, healed lesions in lungs of nonimmunosuppressed rabbit (No 14557) 4 months following intra-arterial injection of *N asteroides* GUH-2. B: Kidney of same rabbit without lesions. C: Lung of rabbit (No 20470) killed by CO_2 inhalation 3 days following IV injection of *N asteroides* GUH-2, 10^3 CFU, showing parenchymal lesion consisting of alveolar histiocytes and lymphocytes with rare polymorphonuclear leukocytes in a micronodular response (HPS, ×250). D: Photomicrograph of same lesion (HPS, ×1250, oil).

E, Table II) were injected in the marginal ear vein with 1 ml of 10^3 CFU *N asteroides* GUH-2. These rabbits had been pretreated 72 hours before the bacterial challenge with 6-methylprednisolone, 30 mg/kg, IM. All four rabbits died between 8 and 11 days after the bacterial challenge (group E, Table II). At necropsy, although no ocular lesions were noted, the internal organs had numerous visible lesions that were indicative of the disseminated disease and that were identical grossly and histologically to those shown in Figs 10 and 11.

Microbiologic Studies.—Selected samples of internal organs were weighed, and a 1:10 wt/vol dilution made with sterile 0.85% saline. The tissue was homogenized, diluted in saline, and spread on the surface of BHI (Difco) and Columbia CNA (Difco) agar plates. The plates were then incubated at 34° C for 3 days.

The culture plates revealed a pure growth of *N asteroides* that was identified by typical colony morphology and by Gram's staining and acid-fast staining. These studies were carried out to demonstrate that the

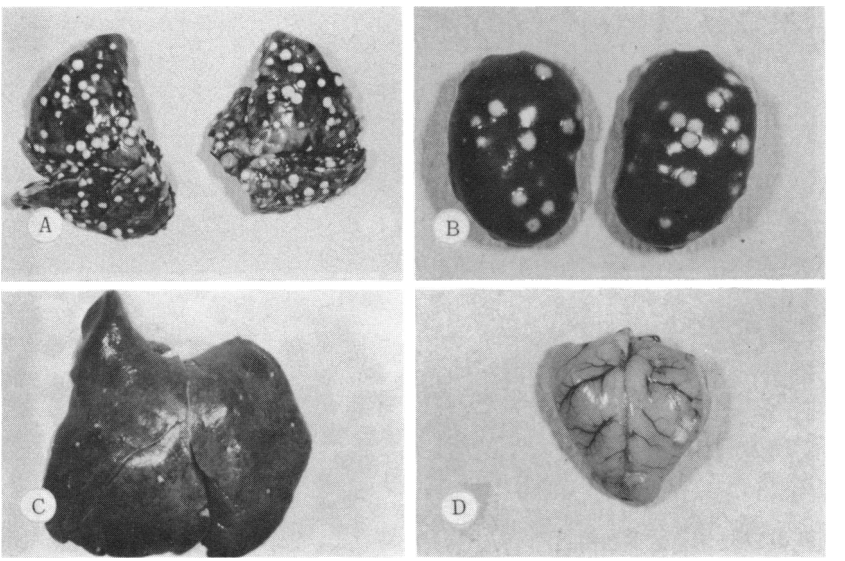


FIGURE 10

A: Lungs of rabbit (No. 12792) immunosuppressed with 6-methylprednisolone, 30 mg/kg, and infected intra-arterially with *N asteroides* GUH-2. Countless large creamy-white abscesses are noted throughout lung tissue. Animal died 21 days after nocardial challenge. B: Kidneys of same rabbit. Numerous abscesses are present. C: Liver of same rabbit. Numerous small abscesses are seen. D: Brain of same rabbit. Brain abscess is noted at periphery of one cerebral hemisphere.

organisms recovered were the same as those injected. No attempt was made to study the quantitative relationships.

STEROID DOSE-RESPONSE DATA

Experimental Design

Twenty-four rabbits were used in a 28-day survival study to determine the lowest dose of the long-acting steroid, 6-methylprednisolone, that was effective in the development of disseminated disease caused by *N asteroides* GUH-2. Intramuscular 6-methylprednisolone was given at doses of 2.5, 5, 10, and 20 mg/kg 3 days prior to the intravascular injection of 10^3 CFU of *N asteroides* GUH-2. The animals were housed and fed as described previously and observed over a 28-day period for the effects of the disseminated disease.

Results.—The data listed in Table III demonstrate that the 20- and 30-mg/kg level dosages allowed the development of a fatal disease in all

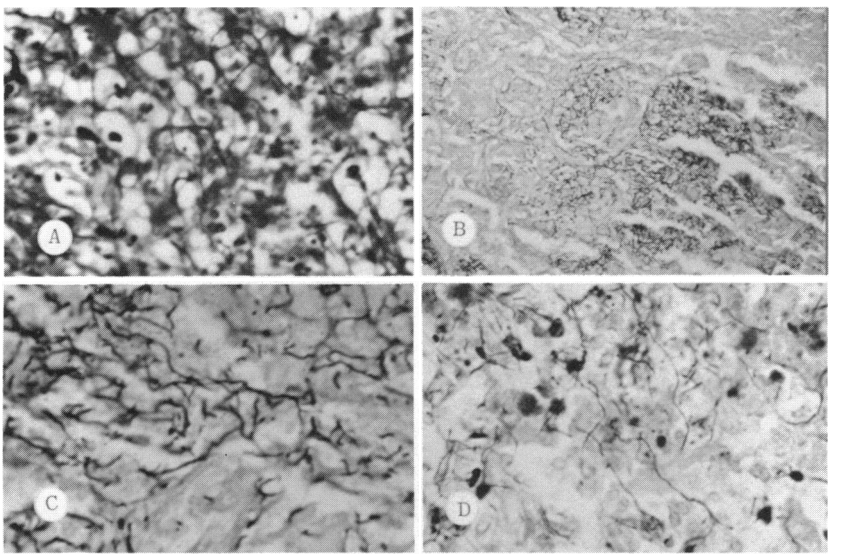
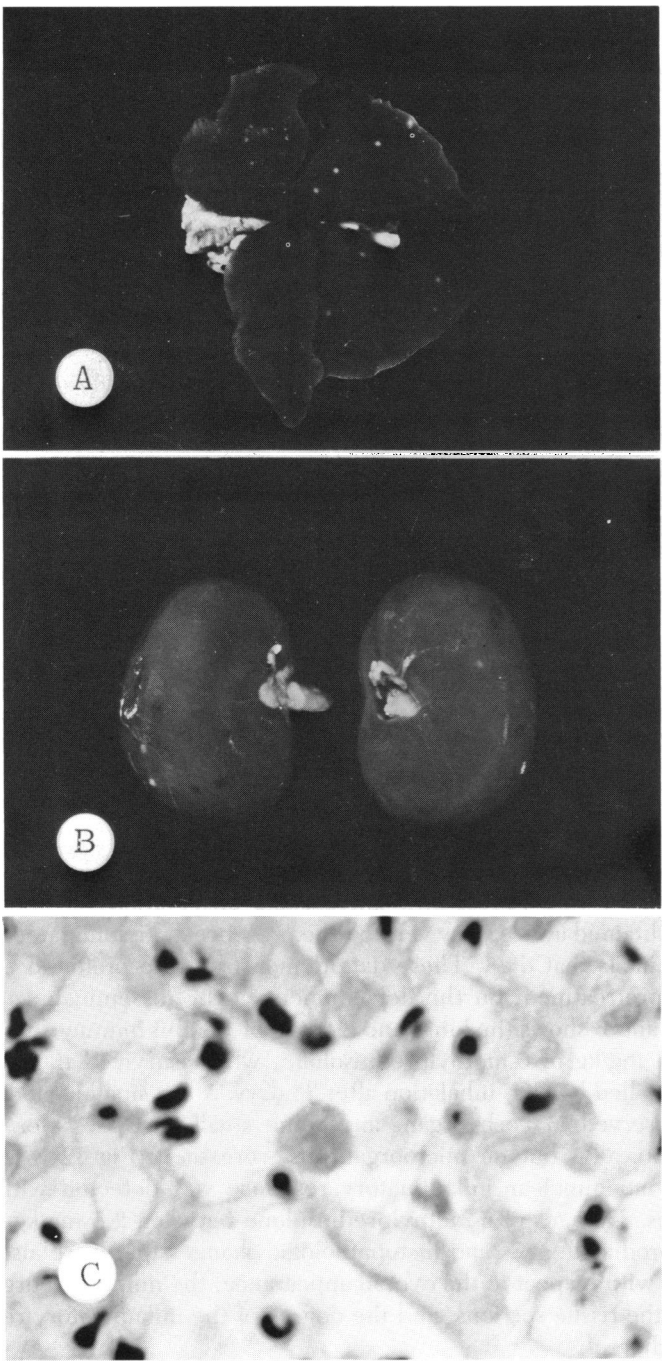


FIGURE 11
A: Lung of rabbit (No 12792) showing tangled, branching, acid-fast nocardial organisms (Fite, ×1250, oil). B: Black-stained organisms within tissue of same specimen (methenamine-silver, ×300). C: Same specimen (methenamine-silver, ×1250, oil). D: Same specimen showing numerous gram-positive branching organisms (Brown-Brenn, ×1250, oil).

the rabbits tested. Gross and microscopic observations of organs and tissues obtained at necropsy verified the presence of disseminated disease in all animals that died. These data demonstrate the profound effect of steroid pretreatment on the development of the disseminated disease. Fig 12A and B shows the lungs and kidneys of a rabbit immunosuppressed with 2.5 mg/kg of 6-methylprednisolone, which survived the infection and was killed by CO_2 inhalation after 34 days. A few small healed lesions were observed in each organ, and some small abscesses were noted microscopically, but no microorganisms were seen (Fig 12C). A more intense mononuclear inflammatory response was detected within the abscesses. Dosages of 6-methylprednisolone between 2.5 mg/kg and 30 mg/kg produced gross and histopathologic changes that were also intermediate with respect to the overall appearance, the number of organisms seen in the tissue sections, and the degree of the inflammatory response or lack thereof.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VARIATIONS OF IMMUNOSUPPRESSIVE REGIMENS

Experimental Design

Seventy-six rabbits were used in a study designed to test the effects of various immunosuppressive medications on the development of the disseminated form of nocardiosis. The immunosuppressive therapy given prior to the intravascular bacterial challenge with 10^3 CFU of *N asteroides* GUH-2 included cyclophosphamide, alone or in combination with either 6-methylprednisolone or hydrocortisone, or methylprednisolone sodium succinate (Solu-Medrol) alone. Hydrocortisone and methylprednisolone sodium succinate are short-acting steroid preparations. Control animals received the immunosuppressive agents followed by intravascular saline 3 days later.

Results.—Sham-Operated Controls. Four rabbits were pretreated with cyclophosphamide, 100 mg/kg, and injected with IV saline as a control for the effect of the cyclophosphamide. As Table IV indicates, all animals (group J) survived the 28-day test period. Eleven rabbits (group L) were then pretreated with both cyclophosphamide, 100 mg/kg, and 6-methylprednisolone, 30 mg/kg, 3 days prior to an intra-arterial saline injection. Six of 11 animals died as a result of the combined immunosuppressive therapy. These animals were not infected with *Nocardia*, yet died from the effects of combined T- and B-cell suppression, possibly from an infectious overgrowth of a preexisting organism. Four rabbits (group P) pretreated with a daily injection of methylprednisolone sodium succinate, 10 mg/kg on 3 consecutive days, received an IV injection of saline, and all four animals survived the 28-day observation period. Four rabbits received a daily injection of 10 mg/kg of methylprednisolone sodium succinate on 3 consecutive days, followed by an IV saline injection, and then by a daily injection of methylprednisolone sodium succinate (10 mg/kg) for 28 days. All four animals survived the 28-day observation period.

Bacterial Injection. Results listed in Table IV show that cyclophosphamide alone (group K) did not significantly enhance the development of the systemic nocardiosis unless this cytotoxic drug was combined with steroids (groups M and N). In those cases, the mortality was 100%. This level of mortality was statistically significant compared with that of the

FIGURE 12

A: Lungs of rabbit (No 16181), immunosuppressed with 6-methylprednisolone, 2.5 mg/kg, and infected intra-arterially with *N asteroides* GUH-2. Rabbit was healthy when killed by CO_2 inhalation 34 days after nocardial challenge. A few small healed lesions are noted. B: Kidneys of same rabbit. A few small healed lesions are noted. C: Photomicrograph of lung of same rabbit showing absence of nocardial organisms (Brown-Brenn, ×1250, oil).

TABLE IV: EFFECTS OF COMBINATIONS OF IMMUNOSUPPRESSIVE AGENTS ON RABBIT MORTALITY IN EXPERIMENTAL NOCARDIOSIS

GROUP	PRETREATMENT MEDICATION	DOSAGE	NO RABBITS	ROUTE OF BACTERIAL CHALLENGE*	10^3 CFU *N ASTEROIDES*	MORTALITY RATE (%)†
J	Cyclophosphamide	100 mg/kg	4	IV	−	0
K	Cyclophosphamide	100 mg/kg	10	8 IV/2 IA	+	10
L	Cyclophosphamide + 6-methylprednisolone	100 mg/kg 30 mg/kg	11	IA	−	55
M	Cyclophosphamide + 6-methylprednisolone	100 mg/kg 10 mg/kg	3	IA	+	100
N	Cyclophosphamide + 6-methylprednisolone	100 mg/kg 30 mg/kg	13	IA	+	100
O	Cyclophosphamide + hydrocortisone	100 mg/kg 300 mg/kg	7	IA	+	71
P	Methylprednisolone sodium succinate	10 mg/kg × 3	4	IV	−	0
Q	Methylprednisolone sodium succinate	10 mg/kg × 3	10	IV	+	10
R	Methylprednisolone sodium succinate	10 mg/kg × 3, 28	4	IV	−	0
S	Methylprednisolone sodium succinate	10 mg/kg × 3, 28	10	IV	+	50

*IA = intra-arterial; IV = intravenous.
†At 28 days.

sham-operated animals ($P = 0.01$). Only one third of the rabbits tested with the 10-mg/kg dose of long-acting steroid (group G, Table III) succumbed to the systemic disease; however, when cyclophosphamide was added to the regimen, the mortality increased to 100% ($P = 0.06$) (group M, Table IV). The organs of the animals receiving both cyclophosphamide and 6-methylprednisolone were identical grossly and histopathologically to those shown in Figs 10 and 11. Cyclophosphamide combined with hydrocortisone was associated with a 71% mortality (group O), and this is a significantly higher mortality than when cyclophosphamide was used alone ($P = 0.01$).

A short course of immunosuppression with a short-term agent such as methylpredisolone sodium succinate followed by the nocardial injection caused only a 10% mortality (group Q) (Fig 13A), while continuous short-term immunosuppression with methylprednisolone sodium succinate resulted in a 50% mortality ($P = 0.05$) (group S) (Fig 13B). These data indicate that the use of short-acting steroids is less likely to produce mortality in rabbits infected with nocardiosis than is the use of long-acting steroids, and that the continuous use of short-term steroids carries a greater risk with an opportunistic infection than discontinued use of these agents. Although cyclophosphamide alone produced only limited mortality from nocardiosis, in combination with steroids it greatly increased the mortality.

IN VITRO DRUG SUSCEPTIBILITIES OF *NOCARDIA ASTEROIDES*

Experimental Design

Minimum inhibitory concentrations (MIC) of four strains of *N asteroides* to four chemotherapeutic agents were determined by agar dilution according to the method of Carroll et al.[136]

The four samples used were (1) GUH-2, as obtained in 1980, (2) GUH-2, as used in 1982, (3) isolated from case 3, and (4) College of American Pathologists (CAP) strain.

Each sample was tested four separate times against each of four chemotherapeutic agents: sulfisoxazole diolamine, sulfadimethoxine, ampicillin, and a sulfamethoxazole-trimethoprim combination (5:1).

Results.—The data in Table V show that *N asteroides* was sensitive to sulfisoxazole diolamine, sulfadimethoxine, and the sulfamethoxazole-trimethoprim combination at MIC levels that were readily attainable in the bloodstream at the doses recommended by the manufacturer.

Each of the GUH-2 samples (1980 and 1982) showed sensitivity to ampicillin at the attainable MIC levels, but the CAP strain and the strain

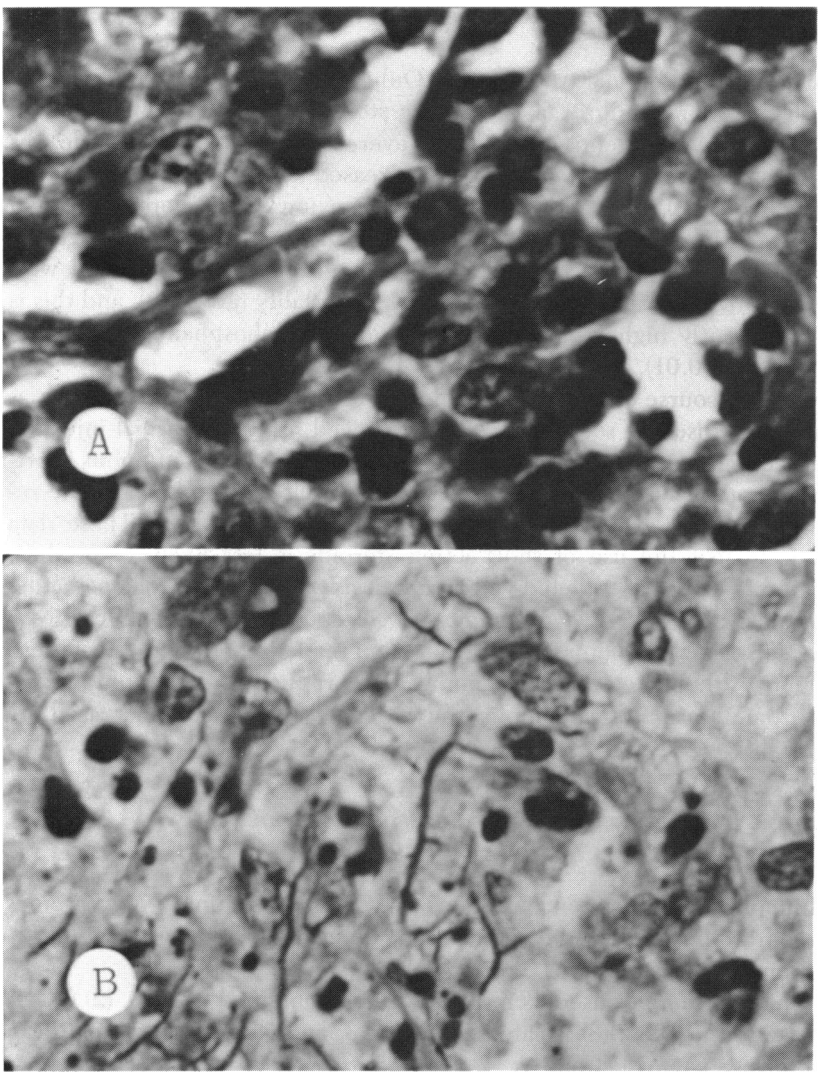


FIGURE 13

A: Lungs of rabbit (No 20455), immunosuppressed with methylprednisolone sodium succinate, 10 mg/kg, 3 consecutive days and then infected IV with 10^3 CFU *N asteroides* GUH-2. Rabbit died 11 days after nocardial challenge. Multiple lung abscesses are shown, with histiocytes and lymphocytes; polymorphonuclear leukocytes were located in necrotic centers of abscesses. No organisms were seen (Brown-Brenn, ×1250, oil). B: Lungs of rabbit (No 20451), immunosuppressed with methylprednisolone sodium succinate, 10 mg/kg, 3 consecutive days, infected IV with 10^3 CFU *N asteroides* GUH-2, and then given daily IM injection of methylprednisolone sodium succinate, 10 mg/kg, for 28 days. Rabbit survived 28-day test period. Severe necrotizing inflammatory reaction with abortive granuloma formation is seen at pheriphery. Many branching forms are seen in necrotic center (Brown-Brenn, ×1250, oil).

TABLE V: IN VITRO DRUG SUSCEPTIBILITIES OF *NOCARDIA ASTEROIDES*

	SULFAMETHOXAZOLE + TRIMETHOPRIM			SULFISOXAZOLE DIOLAMINE		SULFADIMETHOXINE		AMPICILLIN	
	CONCENTRATION (μg/cc)		NO TESTS SENSITIVE/ TOTAL	CONCEN-TRATION (μg/cc)	NO TESTS SENSITIVE/ TOTAL	CONCEN-TRATION (μg/cc)	NO TESTS SENSITIVE/ TOTAL	CONCEN-TRATION (μg/cc)	NO TESTS SENSITIVE/ TOTAL
A	10.0	0.5	0/16	22.5	0/16	10.5	0/16	5.0	0/16
B*	20.0	1.0	10/16	45.0	8/16	21.0	4/16	10.0	0/16
C*	40.0	2.0	16/16	90.0	16/16	42.0	16/16	20.0	0/16
D*	80.0	4.0	16/16	180.0	16/16	84.0	16/16	40.0	6/16
E	160.0	8.0	16/16	360.0	16/16	168.0	16/16	80.0	10/16

*Concentrations attainable in bloodstream.

isolated from case 3 were sensitive only at levels too high to be attainable in the bloodstream. Other in vitro studies have shown comparable results.[127,128,137-139]

EFFECT OF CHEMOTHERAPEUTIC AGENTS ON EXPERIMENTAL RABBIT NOCARDIOSIS

Experimental Design

Forty-five rabbits were used in a study designed to test the effects of various chemotherapeutic agents on experimental nocardiosis. Four antimicrobial regimens were used in the study, as outlined in Table VI. Treatment was started 1 day after the intravascular bacterial challenge with 10^3 CFU *N asteroides* GUH-2.

Results.—The results shown in Table VII indicate the following:

6-Methylprednisolone.—Neither ampicillin (group T) nor trimethoprim-sulfamethoxazole (group U) was effective in the treatment of systemic nocardiosis when the animals were immunosuppressed with 6-methylprednisolone; however, oral sulfadimethoxine reduced the mortality from 100% to 50% ($P = 0.003$) (group V). Fig 14A and B shows the lungs and kidneys of a sulfadimethoxine-treated rabbit that died 16 days following the nocardial challenge. The lesions were smaller than those in animals not receiving antimicrobial treatment.

6-Methylprednisolone-Cyclophosphamide.—The ampicillin-sulfisoxazole combination was not effective in reducing mortality in the doubly immunosuppressed rabbits (group X), but the grossly visible lesions were much smaller than those in animals with identical immunosuppression

TABLE VI: ANTIMICROBIAL REGIMENS

ANTIMICROBIAL REGIMEN NO	ANTIMICROBIAL AGENT	DAILY DOSAGE (FIRST 3 DAYS)	CONTINUOUS DAILY DOSAGE THEREAFTER	ROUTE
1	Ampicillin (Principen, Squibb)	30 mg/kg	10 mg/kg	Oral
2	Trimethoprim-sulfamethoxazole combination (Bactrim, Roche)*	40 mg/kg	30 mg/kg	Oral
3	Sulfadimethoxine (Albon, Roche)	50 mg/kg	25 mg/kg	Oral
4	Ampicillin (Polycillin-N, Bristol)	170 mg/kg	170 mg/kg	Intramuscular
	Sulfisoxazole (Gantrisin, Roche)	800 mg/kg	200 mg/kg	Intramuscular

*Each 5 ml of this oral suspension contains 40 mg of trimethoprim and 200 mg of sulfamethoxazole.

TABLE VII: MORTALITY OF IMMUNOSUPPRESSED RABBITS FOLLOWING CHALLENGE WITH *NOCARDIA ASTEROIDES* GUH-2, AND TREATMENT WITH ANTIMICROBIAL AGENTS

GROUP	PRETREATMENT MEDICATION	DOSAGE	NO RABBITS	ROUTE OF BACTERIAL CHALLENGE*	10^3 CFU *N ASTEROIDES*	ANTIMICROBIAL REGIMEN	MORTALITY RATE (%)†
T	6-Methylprednisolone	30 mg/kg	6	IA	+	1 (Oral ampicillin)	100
U	6-Methylprednisolone	30 mg/kg	5	IA	+	2 (Oral trimethoprim-sulfamethoxazole)	100
V	6-Methylprednisolone	30 mg/kg	6	IA	+	3 (Oral sulfadimethoxine)	50
W	6-Methylprednisolone + cyclophosphamide	30 mg/kg 100 mg/kg	3	IA	−	4 (IM ampicillin, sulfisoxazole)	0
X	6-Methylprednisolone + cyclophosphamide	30 mg/kg 100 mg/kg	9	IA	+	4 (IM ampicillin, sulfisoxazole)	100
Y	Methylprednisolone sodium succinate	10 mg/kg/d × 3 d, 28 d	8	IV	+	2 (Oral trimethoprim-sulfamethoxazole)	12
Z	Methylprednisolone sodium succinate	10 mg/kg/d × 3 d, 28 d	8	IV	+	3 (Oral sulfadimethoxine)	50

*IA = intra-arterial; IV = intravenous.
†At 28 days.

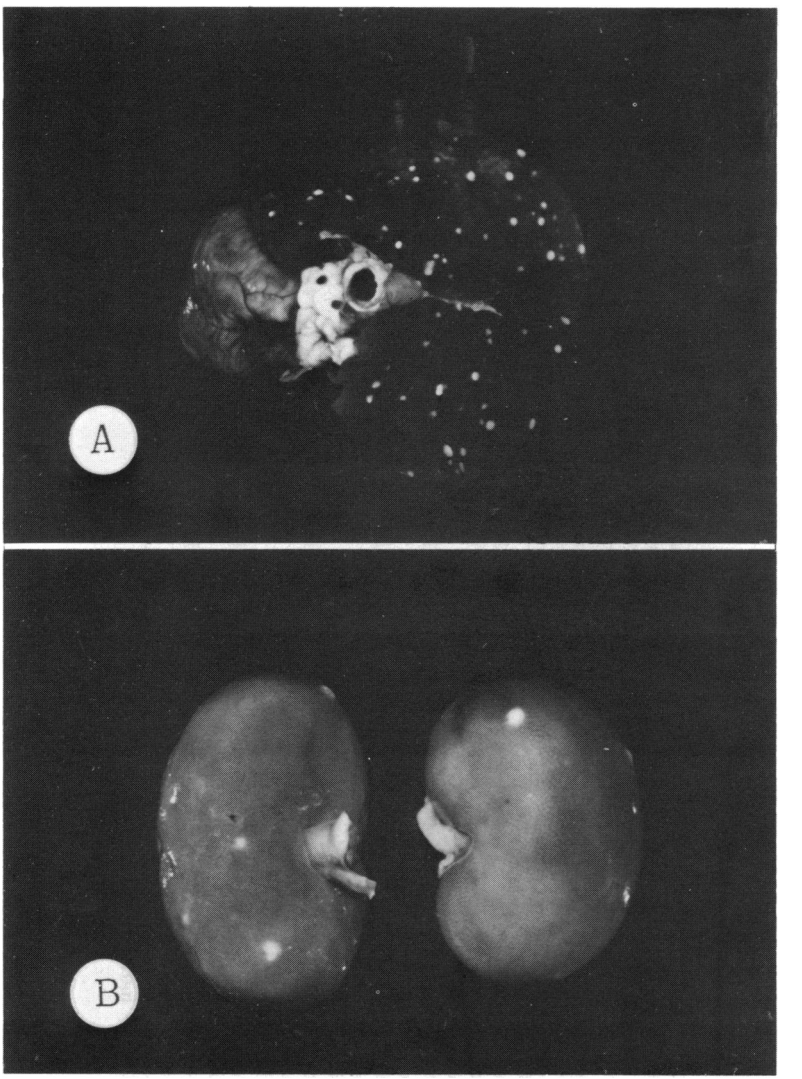


FIGURE 14

A: Lungs of rabbit (No 16691), immunosuppressed with 6-methylprednisolone, 30 mg/kg, and infected with *N asteroides* GUH-2. Animal was treated with sulfadimethoxine (regimen 3). Tissues show fewer and smaller lesions than were evident without antimicrobial treatment. Rabbit died 16 days after nocardial challenge. B: Kidneys of same rabbit.

but without antimicrobial treatment (Fig 15). Fewer abscesses were visible microscopically in these animals than in nonchemotherapeutically treated rabbits. The abscesses themselves looked identical in terms of the lack of a cellular response, but within the abscesses, organisms were much harder to locate. Antimicrobial treatment with ampicillin and sulfisoxazole in noninfected rabbits immunosuppressed with 6-methylprednisolone, 30 mg/kg, and cyclophosphamide, 100 mg/kg, was not harmful, and there was no mortality (group W).

Methylprednisolone Sodium Succinate.—Fifty percent of the animals treated with daily methylprednisolone sodium succinate died after the nocardial challenge (Table IV, group S). Sulfadimethoxine was ineffective as an antimicrobial agent in reducing mortality (Table VII, group Z) (Fig 16A); however, the mortality was reduced to 12% when trimethoprim-sulfamethoxazole therapy was used ($P = 0.1$) (Table VII, group Y) (Fig 16B).

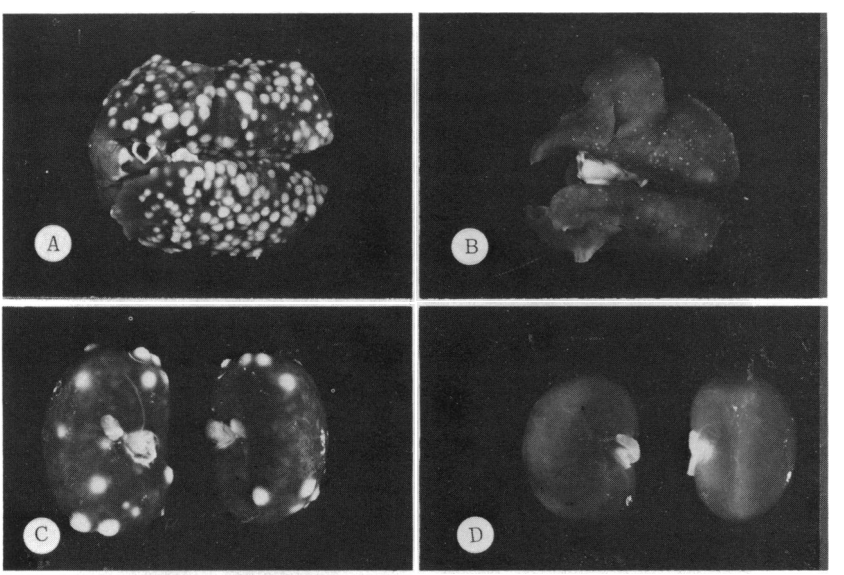
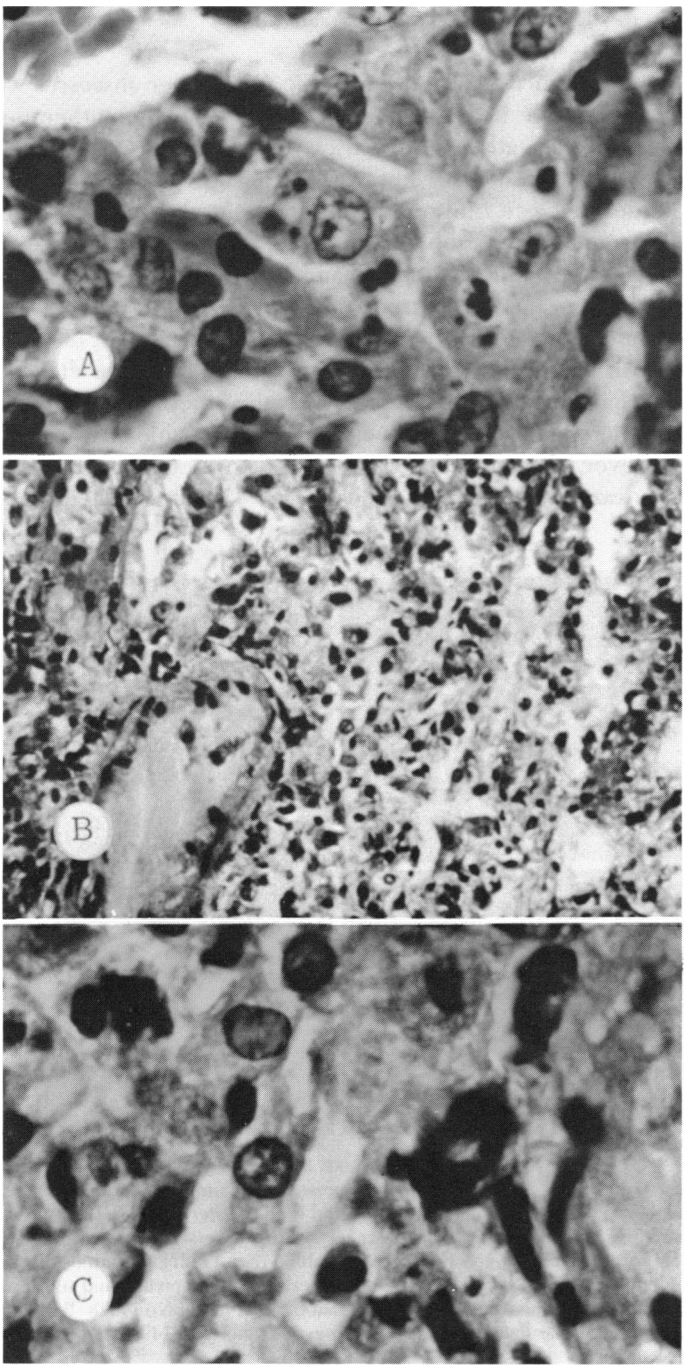


FIGURE 15

A: Lungs of rabbit (No 14928), immunosuppressed with 6-methylprednisolone, 30 mg/kg, and cyclophosphamide, 100 mg/kg, and infected with *N asteroides* GUH-2. No antimicrobial therapy was given. Countless large, creamy-white abscesses are noted. Animal died 12 days after nocardial challenge. B: Lungs of rabbit (No 14927), immunosuppressed and infected identically to No 14928. Antimicrobial therapy with ampicillin and sulfisoxazole was given (regimen 4). Lesions are much smaller than those of rabbit No 14928. Rabbit No 14927 died 11 days after nocardial challenge. C: Kidneys of rabbit No 14928. D: Kidney of rabbit No 14927.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OPHTHALMIC OBSERVATIONS

ANATOMIC STUDIES

Seven animals were used to study the anatomic relationships of the carotid artery and those arterial branches that supply the eye. Two animals were dissected for localization of the carotid arteries in relation to the trachea. The rabbits' carotid arteries, like their human counterparts, were noted to lie on either side of the trachea (Fig 7B).

Sham operations were carried out by carotid puncture in two animals injected with saline for simultaneous visualization of the retinal vessels. Two animals were injected with sodium fluorescein, 0.5 gm (Fluorescite Injection 5%, Alcon Laboratories, Inc), for simultaneous visualization of the retinal vessels using a cobalt-blue filter, and one animal was injected with India ink for simultaneous visualization of the retinal vessels and histologic examination of the eye for the presence of intravascular India ink. These studies demonstrated that substances (saline, fluorescein, and India ink) injected into the carotid artery appeared promptly and were readily observed in the chorioretinal and iris vasculature of the rabbit eye.

OBSERVATION OF EYE LESIONS

Experimental Design

Retinal Lesions. The rabbits' pupils were dilated with a combination of 2.5% phenylephrine hydrochloride (Neo-Synephrine, Winthrop), 1% tropicamide (Mydriacyl, Alcon), and 2% homatropine hydrobromide (Isopto-Homatropine, Alcon). One drop of each medication was instilled into the conjunctival sac of each eye, and prompt, wide pupillary dilation was evident within 20 to 30 minutes. The retina of each intra-arterially injected rabbit was observed using an indirect ophthalmoscope, and selected animals were photographed using a retinal camera (Kowa, Keeler

FIGURE 16

A: Lungs of rabbit (No 20723), immunosuppressed with methylprednisolone sodium succinate, 10 mg/kg, 3 consecutive days, infected IV with 10^3 CFU *N asteroides* GUH-2, and then given a daily IM injection of methylprednisolone sodium succinate, 10 mg/kg. Rabbit was treated with sulfadimethoxine (regimen 3). Rabbit died 19 days after bacterial challenge. Photomicrograph shows histiocytes that are phagocytizing necrotic debris. No organisms are seen (Brown-Brenn, ×1250, oil). B: Lungs of rabbit (No 20730), immunosuppressed and infected identically to rabbit No 20723 but treated with trimethoprim-sulfamethoxazole (regimen 2). Rabbit died 18 days after bacterial challenge. Photomicrograph shows diffuse pneumonia with abscess formation and polymorphonuclear leukocytes. No organisms are seen (Brown-Brenn, ×250). C: Same lesion shown in Fig 16B (Brown-Brenn, ×1250, oil).

Optical) and color slide film (Kodachrome 64, Eastman Kodak Company). The rabbit eyes were examined for the presence, size, number, appearance, distribution, and growth rate of ipsilateral and contralateral nocardial retinal lesions.

Iris Lesions. The irides of selected animals were photographed using a 35 mm camera with an extender lens capable of one-to-one reproduction and color slide film (Kodachrome 25, Eastman Kodak Company).

Results.—Intravenous Injection. Sixty-two animals met the following criteria: (1) They received an IV injection of *N asteroides* GUH-2, 10^3 CFU. (2) They underwent at least two external ocular examinations, one at 2 days after injection and a second examination 6 to 14 days after injection. Dilated fundus examinations were not performed when the *N asteroides* was injected IV because of the expected low yield of chorioretinal lesions. The irides, however, were observed, and an iris lesion was present in 1 of these 62 animals (1.6%) (Fig 17).

Intra-Arterial Injection. Seventy-six animals met the following criteria: (1) They received an intra-arterial injection of *N asteroides* GUH-2, 10^3 CFU. (2) They underwent at least two complete ocular examinations including dilated funduscopy 2 days after injection and a second examination 6 to 14 days after injection. The data from these 76 rabbits are shown in Table VIII. The 76 rabbits were divided into nine separate groups based on the pretreatment medications.

Saline. Nonimmunosuppressed animals that were given intracarotid artery injections of *N asteroides* GUH-2 showed multiple small retinal infiltrates that developed in the first few days after injection, enlarged slightly over the next few weeks, and finally healed with scarring (Fig 18A, B, and C). Histopathologic examination of the retinas of these animals showed a distinctive pattern. In the areas of chorioretinal scarring, no organisms were noted; the retina was adherent to the underlying choroid while the remainder of the retina was artifactitiously detached. The adherent retina and underlying choroid were disorganized in contrast to the adjacent retina and choroid, which were normal. The pigment epithelium was absent in the scarred area (Fig 18D).

A small iris lesion was noted in one nonimmunosuppressed rabbit (Fig 19). This lesion eventually healed by scarring and vascularization.

Cyclophosphamide. In animals pretreated with cyclophosphamide alone, neither chorioretinal nor iris lesions developed.

6-Methylprednisolone, 30 mg/kg. The chorioretinal lesions in the animals immunosuppressed with 6-methylprednisolone, 30 mg/kg, appeared as multiple, round, creamy-white infiltrative lesions. Against the red background of the retina, they resembled *Staphylococcus* colonies on a

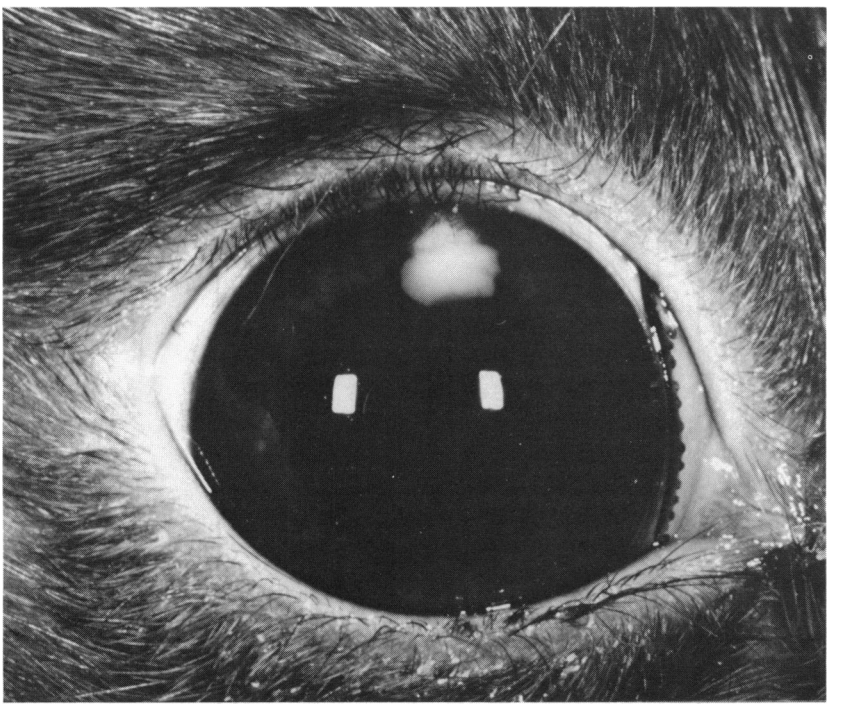

FIGURE 17
Typical superior iris abscess in rabbit No 16874, immunosuppressed with 6-methylprednisolone, 30 mg/kg, 10 days following IV injection of *N asteroides* GUH-2. Animal died 11 days following nocardial challenge.

blood agar plate[119] or fresh argon laser spot lesions (Fig 20A). These lesions usually appeared on the second day after injection, but in some cases were present on the first day after injection. In 55% of the cases in which lesions ultimately developed, additional lesions developed between the second and seventh days. Once the lesions appeared, however, they enlarged, coalesced, and resembled larger, more irregular, white patchy areas similar to those seen immediately after retinal cryosurgery (Fig 20B and C). Most of the chorioretinal lesions were posterior to the equator and inferior to the optic nervehead and horizontal vessels. Iris lesions were noted in 5.7% of all immunosuppressed rabbits (Table VII) and were usually located superiorly. Fig 21A and B shows enlargement of an iris lesion. Eyelid lesions were also seen. Fig 21C and D shows enlargement and coalescence of chorioretinal lesions in the same animal,

TABLE VIII: OPHTHALMIC LESIONS IN EXPERIMENTAL NOCARDIOSIS*

PRETREATMENT MEDICATION	DOSE	NO RABBITS	NO (%) WITH IPSILATERAL RETINAL LESIONS	AVERAGE NO OF INITIAL RETINAL LESIONS	DISTRIBUTION OF RETINAL LESIONS	NO (%) WITH CONTRALATERAL LESIONS	NO (%) WITH IRIS LESIONS
Saline	. . .	6	5 (83)	2.3	Mostly inferior	0 (0)	1 (16.7)
Cyclophosphamide	100 mg/kg	4	0 (0)	0	. . .	0 (0)	0 (0)
6-Methylprednisolone	30 mg/kg	30	19 (63)	7.6	Mostly inferior	1 (3.3^{r})†	2 (6.7)
6-Methylprednisolone	10 mg/kg	4	1 (25)	6.2	Mostly inferior	1 (25.0^{i})‡	0 (0)
6-Methylprednisolone	5 mg/kg	3	3 (100)	6.3	Mostly inferior	1 (33.3^{r})†	1 (33.3)
6-Methylprednisolone	2.5 mg/kg	3	2 (67)	0.7	Exclusively inferior	0 (0)	0 (0)
Cyclophosphamide + hydrocortisone	100 mg/kg 300 mg/kg	5	3 (60)	3.6	Mostly inferior	0 (0)	0 (0)
6-Methylprednisolone + cyclophosphamide	10 mg/kg 100 mg/kg	3	3 (100)	3.7	Mostly inferior	0 (0)	0 (0)
6-Methylprednisolone + cyclophosphamide	30 mg/kg 100 mg/kg	18	11 (61)	4.0	Mostly inferior	1 (5.6^{i})‡	1 (5.6)
All groups	. . .	76	47 (62)	5.1	Mostly inferior	4 (5.3)	5 (6.6)

*Intra-arterial injections.
†r = retina.
‡i = iris.

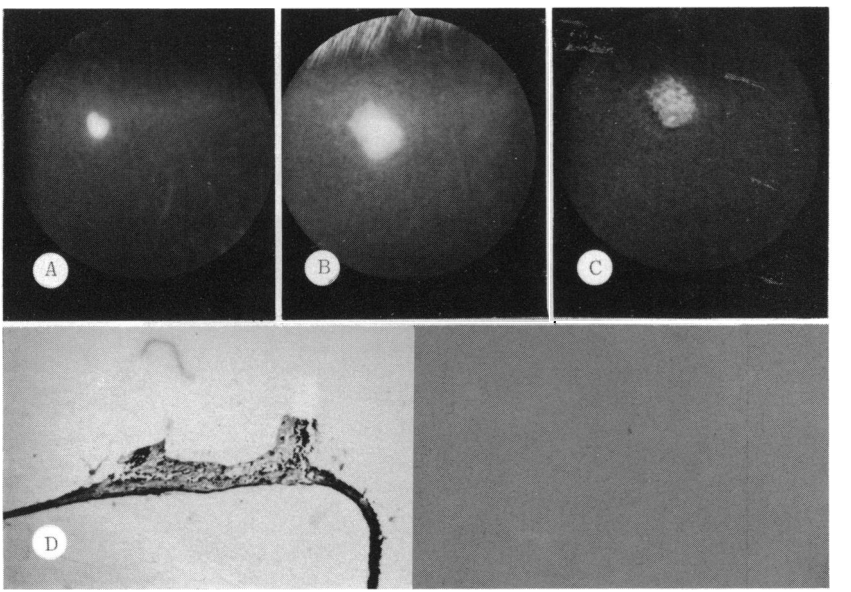

FIGURE 18

A: Retinal photograph of nonimmunosuppressed rabbit No 16192, 2 days after intra-arterial injection of *N asteroides* GUH-2. B: Same lesion after 18 days. Lesion is enlarged. C: Same lesion after 42 days. Lesion has completely healed. D: Lesion shown in Fig 18C, showing retina adherent to underlying choroid. Remainder of retina is artifactitiously detached. Adherent retina and underlying choroid are disorganized, in contrast to adjacent retina and choroid, which were normal (not shown). Pigment epithelium is absent in involved area (HPS, ×80).

and Fig 21E shows the microscopic appearance of these chorioretinal abscesses.

Trimethoprim-sulfamethoxazole was previously noted in this study to be ineffective against experimental nocardiosis when the animals were immunosuppressed with 6-methylprednisolone, 30 mg/kg (group U). Iris and retinal lesions were not affected by this antimicrobial therapy (Fig 22).

6-Methylprednisolone, 10 mg/kg. A 10-mg/kg dosage produced retinal lesions in only one of the four animals. The lesions grew and coalesced in the same manner as those in animals receiving the 30-mg/kg dosage.

6-Methylprednisolone, 5 mg/kg. A 5-mg/kg dosage of 6-methylprednisolone produced retinal lesions in all three of the animals injected intra-arterially. Four lesions were noted initially in rabbit No 16180. Later, by 30 days after infection, these lesions spread diffusely throughout the choroid and retina, producing a retinal detachment (Fig 23A). By 70 days,

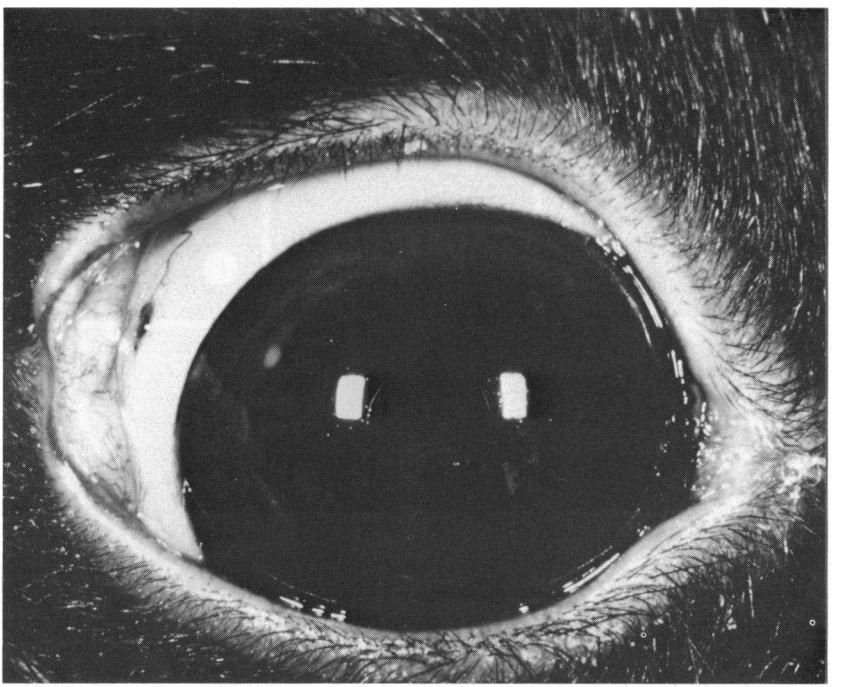

FIGURE 19
Iris of nonimmunosuppressed rabbit (No 14557), 14 days after challenge with *N asteroides* GUH-2. Small lesion is noted at 9:30-o'clock position at pupillary border.

the infectious process was resolving (Fig 23B), and by 180 days, only minor scarring persisted (Fig 23C).

6-Methylprednisolone, 2.5 mg/kg. A dosage of 2.5 mg/kg yielded retinal lesions in two thirds of the rabbits injected intra-arterially. The lesions grew slowly but finally resolved with scarring (Fig 24).

Cyclophosphamide-Hydrocortisone. When cyclophosphamide was combined with hydrocortisone, a slower development of the chorioretinal lesions was noted than when cyclophosphamide was combined with 6-methylprednisolone. Fig 25A shows a nonrhegmatogenous retinal detachment as a result of a nocardial abscess in the choroid, 11 days after infection. Superior neovascularization was present. The retinal detachment was presumably caused by inflammation in the choroid and retina and may represent the body's attempt to control the infection. With potent, long-term immunosuppression, inflammatory reaction to the *Nocardia* was essentially absent, and therefore, no concomitant retinal detachment was noted. Fig 25B shows resolution of the retinal detachment

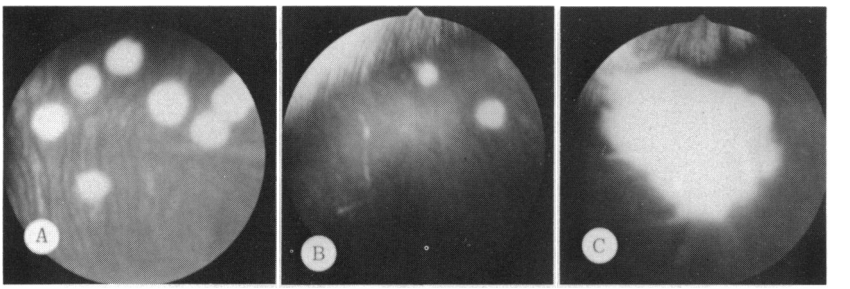

FIGURE 20

A: Retina of rabbit No 16684 (immunosuppressed with 6-methylprednisolone, 30 mg/kg, and infected with an ipsilateral intracarotid injection of *N asteroides* GUH-2), 2 days after bacterial challenge. Multiple creamy-white abscesses resemble staphylococcal colonies on blood agar plate[119] or acute argon laser photocoagulation lesions. B: Retina of rabbit No 14555 (immunosuppressed with 6-methylprednisolone, 30 mg/kg, and cyclophosphamide, 100 mg/kg), 2 days after nocardial challenge. Two chorioretinal abscesses are noted. C: Retina of rabbit No 14555 7 days after nocardial challenge, showing enlargement and coalescence of the two lesions. It now resembles an acute retinal cryosurgery lesion.

within 1 week and a retinal hemorrhage adjacent to the abscess. By 37 days (Fig 25C) the hemorrhage resolved and the lesion enlarged. By 77 days (Fig 25D) early vascularization of the abscess was present. The animal was then killed by CO_2 inhalation, and a large retinal abscess was noted (Fig 25E).

6-Methylprednisolone/Cyclophosphamide, 100 mg/kg. When cyclophosphamide was combined with 6-methylprednisolone (at either the 10- or 30-mg/kg level), the retinal lesions were identical to those seen in animals treated with 6-methylprednisolone, 30 mg/kg, alone (Fig 20B and C).

CHORIORETINAL LESION DEVELOPMENT

Experimental Design

Photographs of selected rabbits with chorioretinal lesions were examined. Lesions that appeared on serial photographs over several time periods were assembled. Representative photographs from rabbits pretreated with saline or with different doses and combinations of immunosuppressive medications, with or without antimicrobial therapy, were projected at a fixed distance onto plain white paper. The projected lesions were traced onto the paper, and the areas of the lesions were measured in relative units, using a compensating polar planimeter (No 620010, Keuffel & Essen Company, Morristown, NJ).

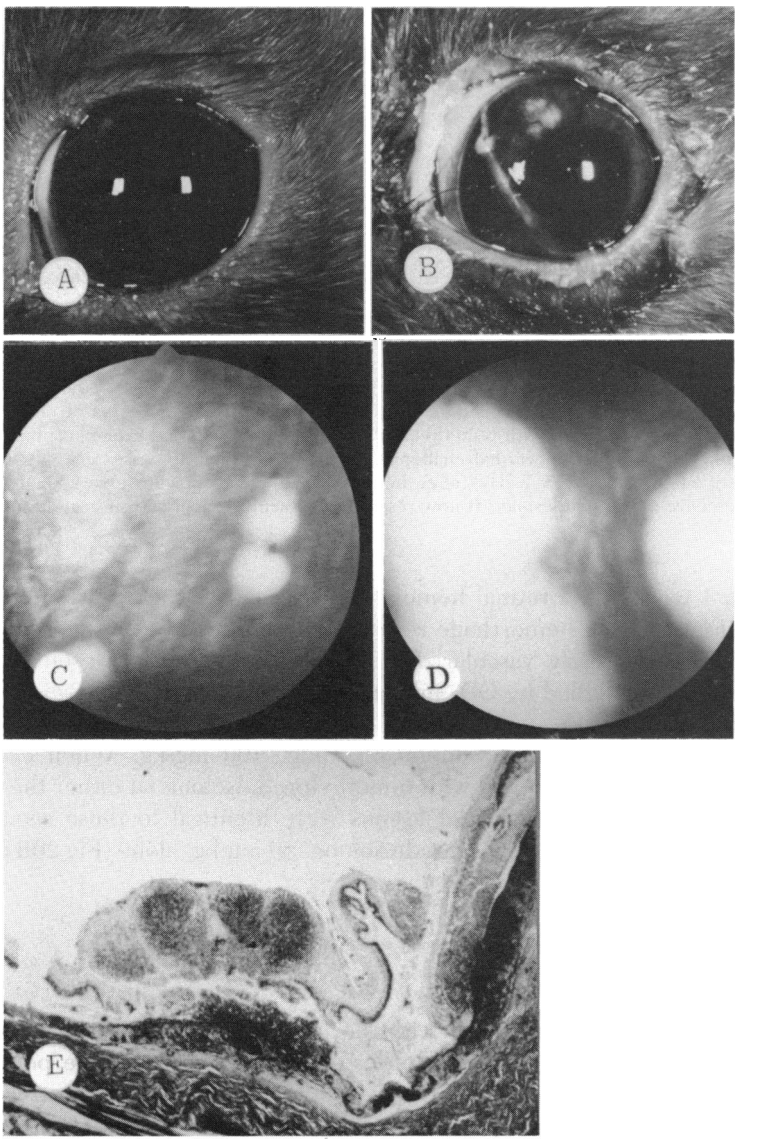

FIGURE 21

A: Upper eyelid abscess and superior iris abscess in rabbit No 12792 (immunosuppressed with 6-methylprednisolone, 30 mg/kg), 7 days after intra-arterial nocardial challenge. B: Same rabbit 12 days after nocardial challenge, showing marked enlargement of iris lesion. C: Retina of same rabbit 2 days after nocardial challenge. D: Retina of same rabbit 12 days after nocardial challenge. E: Same rabbit showing chorioretinal abscesses (Brown-Brenn, ×25).

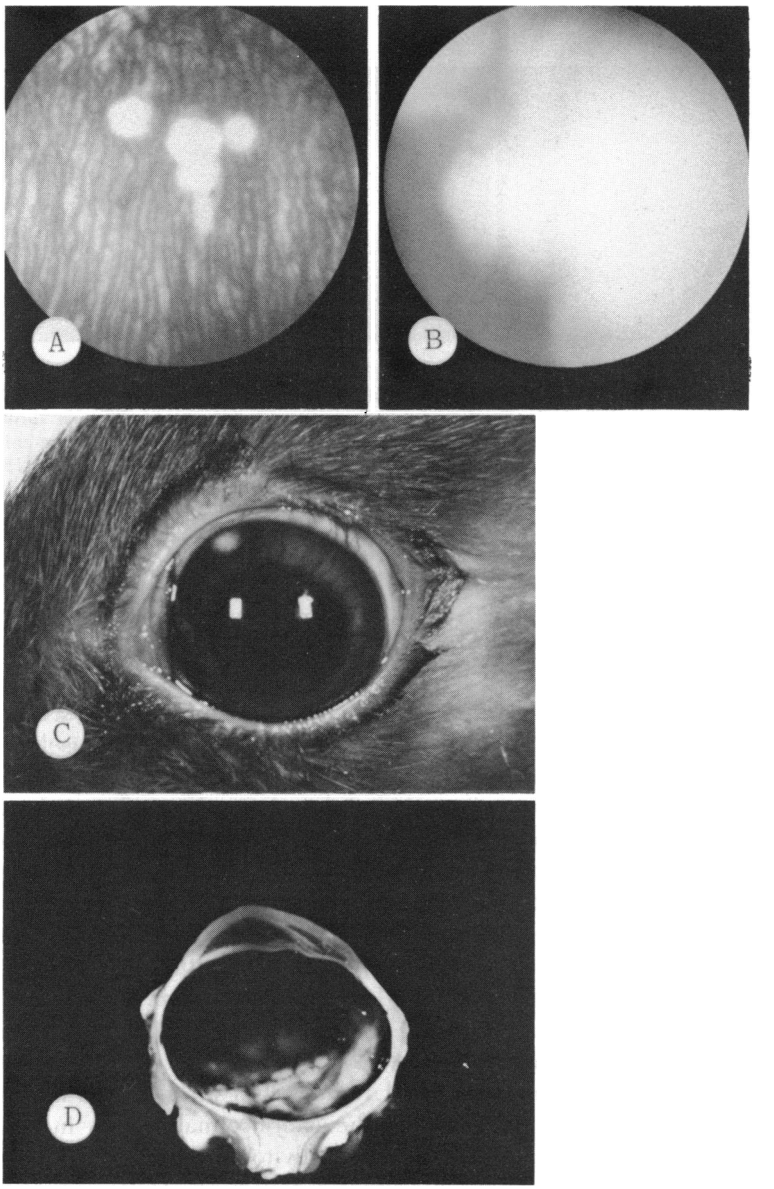

FIGURE 22

A: Retina of rabbit No 16684 (immunosuppressed with 6-methylprednisolone, 30 mg/kg), 2 days after nocardial challenge. Rabbit was being treated with oral trimethoprim-sulfamethoxazole (regimen 2). Multiple small lesions are seen. B: Same lesions at 7 days. Large retinal detachment from massive choroidal abscess is noted. C: Same animal, showing superior iris abscess. D: Gross pathologic appearance of same eye 9 days after infection, showing massive confluent chorioretinal lesion.

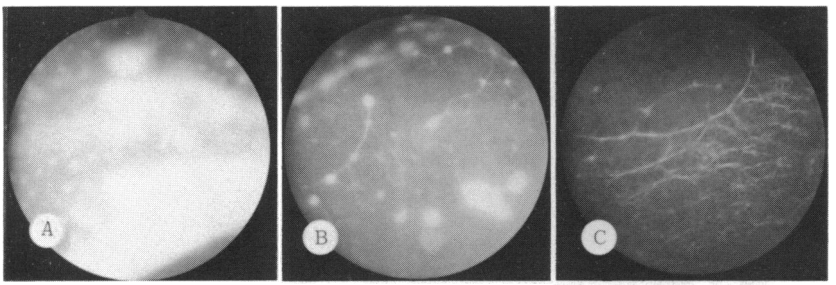

FIGURE 23

A: Retina of rabbit No 16180 (immunosuppressed with 6-methylprednisolone, 5 mg/kg), 30 days after nocardial challenge. Lesions have coalesced and spread diffusely throughout retina and choroid, producing nonrhegmatogenous retinal detachment. B: Same animal 70 days after nocardial challenge, showing resolution of infection. C: Same animal 180 days after nocardial challenge, showing complete resolution and healing.

Results.—Steroid Immunosuppression. Fig 26A, B, C, and D shows the enlargement of a chorioretinal lesion over a 21-day period after nocardial injection in a rabbit (No 13456) immunosuppressed with 6-methylprednisolone, 30 mg/kg. (Fig 26E, F, and G shows the histopathologic appearance of this lesion.) Fig 24 shows, over a 6-month period, the chorioretinal lesion enlargement and evolution, with eventual scarring, in a rabbit (No 16182) immunosuppressed with 6-methylprednisolone, 2.5 mg/kg. Fig 27 shows the evolution of a lesion in a rabbit (No 16689) immunosuppressed with 6-methylprednisolone, 30 mg/kg, and treated with sulfadimethoxine. Fig 18A, B, and C shows the evolution of a lesion in a rabbit (No 16192) pretreated with saline. Fig 28 shows the comparative lesion growth rates for these rabbits, with lesion area plotted (in relative planimeter units) against time (in days). The curve for rabbit No 13456 suggests the growth curve of bacteria in culture.

Steroid and Cyclophosphamide Immunosuppression. Fig 29 shows the enlargement of the chorioretinal lesions in a rabbit (No 14926) immunosuppressed with 6-methylprednisolone, 30 mg/kg, and cyclophosphamide, 100 mg/kg. The rabbit died 10 days after intra-arterial infection.

Fig 30 shows the evolution of the chorioretinal lesions in a rabbit immunosuppressed and infected identically to rabbit No 14926 but treated with IM ampicillin and sulfisoxazole diolamine. These antimicrobial agents have produced marked suppression of growth of the retinal lesions. The rabbit died 11 days after infection.

Fig 31 shows the lesion growth rates for the rabbits receiving steroid and cyclophosphamide immunosuppression and shows the profound effect of antimicrobial therapy (ampicillin and sulfisoxazole diolamine).

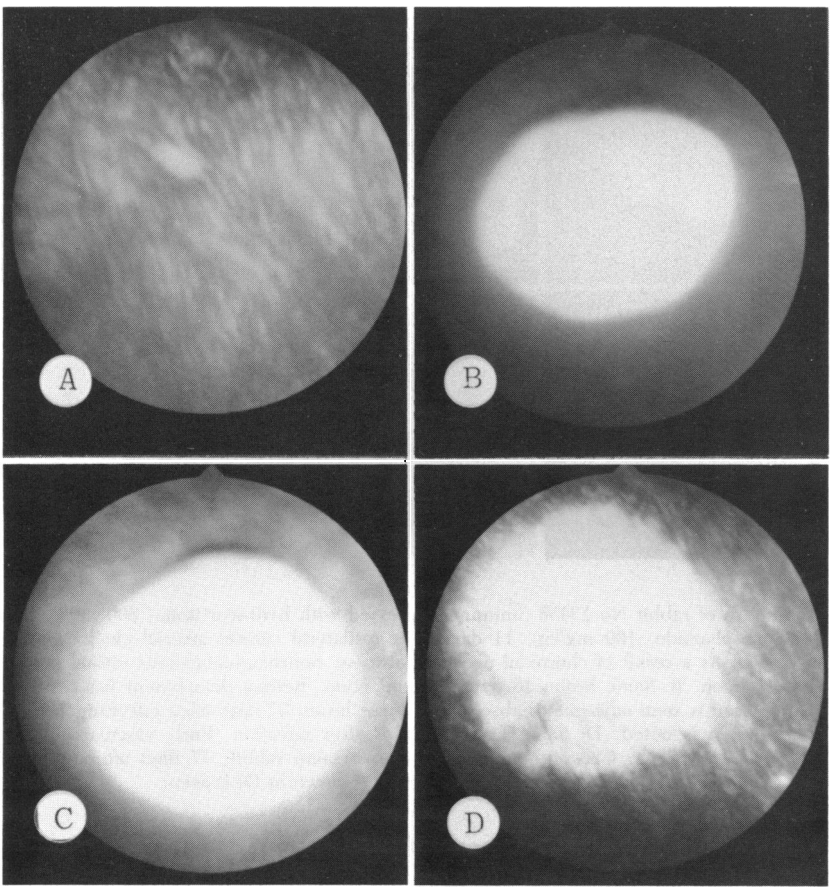

FIGURE 24

A: Retina of rabbit No 16182 (immunosuppressed with 6-methylprednisolone, 2.5 mg/kg), 2 days after nocardial challenge. Small abscess is seen (just above center). B: Same lesion 18 days after nocardial challenge. Slow, progressive enlargement of lesion is noted. C: Same lesion at 30 days. Slight additional growth is seen since day 18. D: Same lesion at 180 days. Lesion has totally regressed, and only chorioretinal scar is seen.

DISCUSSION

The clinical features of the ten previously reported patients with endogenous ocular nocardiosis are given in Table I. These data reveal a mean age of 46 years and a male-to-female ratio of 4:1. Numerous predisposing factors in these patients included (1) a serious underlying disease, (2) a serious injury, (3) organ transplantation, (4) corticosteroid therapy, (5)

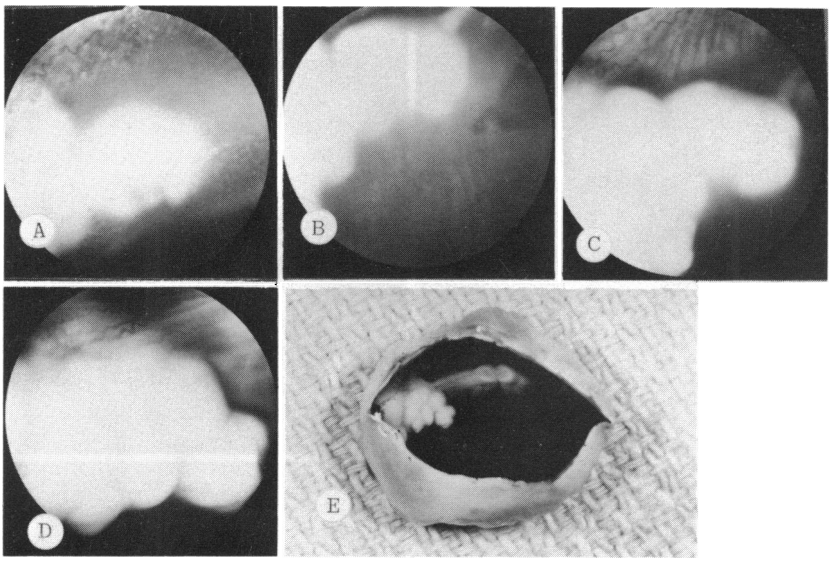

FIGURE 25

A: Fundus of rabbit No 13458 (immunosuppressed with hydrocortisone, 300 mg/kg, and cyclophosphamide, 100 mg/kg), 11 days after ipsilateral carotid arterial challenge with *Nocardia*. As a result of choroidal nocardial abscess, nonrhegmatogenous retinal detachment is seen. B: Same lesion 18 days after infection. Retinal detachment has resolved; hemorrhage is seen adjacent to abscess. C: Same lesion 37 days after infection. Further enlargement is noted. D: Same lesion 77 days after infection. Early vascularization of abscess is noted. E: Gross photograph of eye of same rabbit, 77 days after nocardial challenge. Large retinal abscess (shown in D) is seen.

other immunosuppressive therapy, (6) radiation therapy, and (7) antimicrobial therapy. Of the ten patients previously reported, one had seven predisposing factors[116] and one had none.[117] The median number of predisposing factors was three per patient.

The organisms were recovered from a variety of sources including (1) lung (sputum), (2) eye, (3) orbit, (4) blood, and (5) a nonpulmonary, nonocular abscess. There was a median of two recovery sites per patient.

The ocular symptoms in these patients included blurred vision in five patients, ocular pain in three patients, redness in one patient, and a foreign body sensation in one patient. (Some patients had more than one symptom, and some patients had no symptoms.)

There was no right or left eye predisposition: four of the unilateral cases occurred on the right side, and three of the unilateral cases occurred on the left side. Bilateral ocular involvement was present in three of the ten patients (30%).

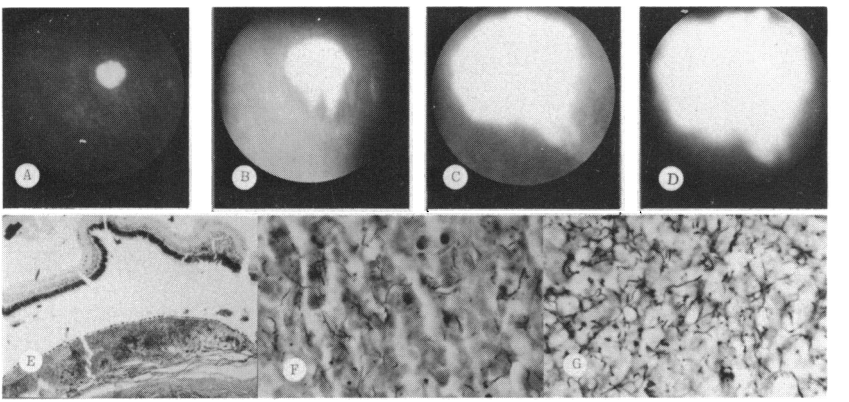

FIGURE 26

A: Retina of rabbit No 13456 (immunosuppressed with 6-methylprednisolone, 30 mg/kg), 2 days after nocardial challenge. Solitary lesion is noted. B: Same lesion 4 days after infection. C: Same lesion 9 days after infection. D: Same lesion 21 days after infection. Rabbit died following photography. E: Lesion seen in D, showing large choroidal abscess (Brown-Brenn, ×60). F: Same lesion showing numerous gram-positive organisms (Brown-Brenn, ×1250, oil). G: Same lesion showing numerous nocardial organisms (methenamine-silver, ×1250, oil).

Of the 13 eyes involved, 10 had a choroidal mass, 3 had a cloudy vitreous, 2 had proptosis, 3 had uveitis, and 1 had cranial nerve involvement (a unilateral sixth nerve palsy and a bilateral seventh nerve palsy), 2 had a nonrhegmatogenous retinal detachment, and 1 had scattered chorioretinal infiltrates (presumably similar to those seen in the rabbits described earlier).

Treatment consisted of enucleation in 4 of the 13 eyes; the remaining patients were treated with a variety of antimicrobials including sulfadiazine (3 patients), cycloserine (3 patients), triple sulfa (2 patients), trimethoprim-sulfamethoxazole (2 patients), and erythromycin, minocycline, cotrimaxazole, sulfisoxazole, penicillin, iodides, and streptomycin (1 each).

The ocular histopathologic findings varied from a granulomatous reaction, to a lymphocytic and polymorphonuclear leukocytic infiltration, to an acute necrotizing chorioretinitis. The organisms were seen histopathologically in the eyes of eight of the nine patients whose eyes were recovered either at the time of enucleation or at autopsy.

Four of nine patients (in whom an outcome was stated) died as a result of disseminated nocardiosis (44%). Of the two nonimmunosuppressed patients, one died. Of the seven immunosuppressed patients, three died.

In the present report, the three patients described are typical of the other reported patients with disseminated endogenous ocular nocardio-

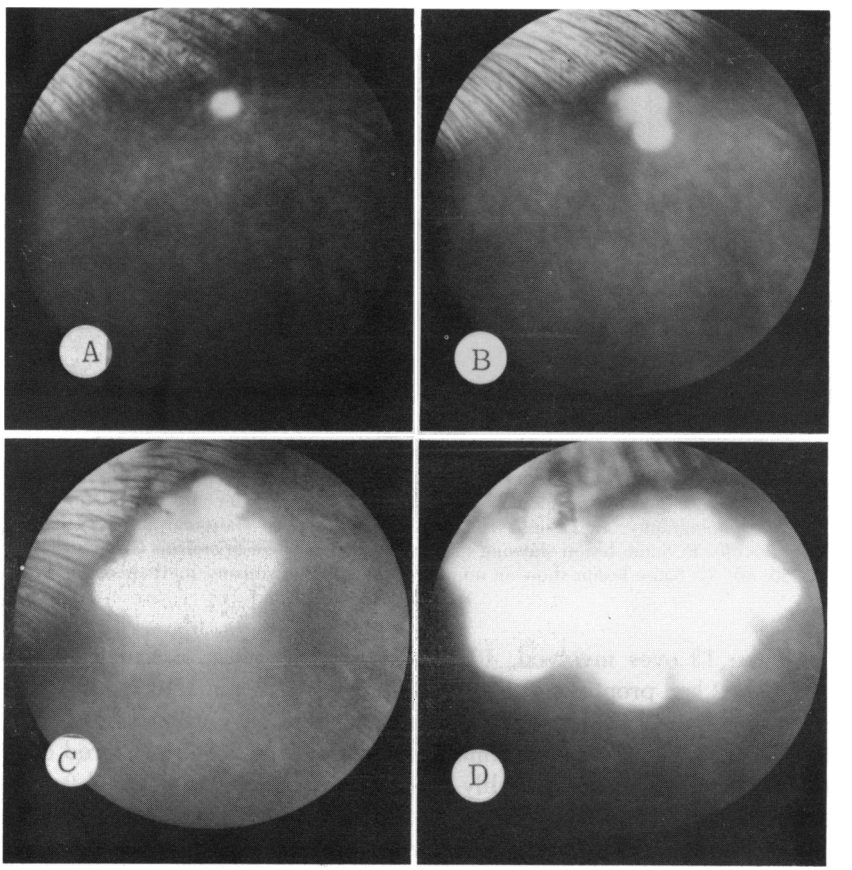

FIGURE 27

A: Retina of rabbit No 16689 (immunosuppressed with 6-methylprednisolone, 30 mg/kg), 2 days after bacterial challenge. Solitary lesion is noted. Animal was being treated with oral sulfadimethoxine (regimen 3). B: Same lesion 7 days after infection. C: Same lesion 20 days after infection. D: Same lesion 35 days after infection. Early vascularization is seen.

sis. The three patients were male, with an average age of 36 years. The first patient had four predisposing factors: Wegener's granulomatosis, renal transplant, corticosteroid therapy, and azathioprine therapy. The second patient had three predisposing factors: paroxysmal nocturnal hemoglobinuria, bone marrow transplantation, and prednisone therapy. The third patient was diabetic. Two of the three patients were immunosuppressed. Disseminated nocardiosis presumably developed in the third patient as a result of exposure to an extremely large number of inhaled

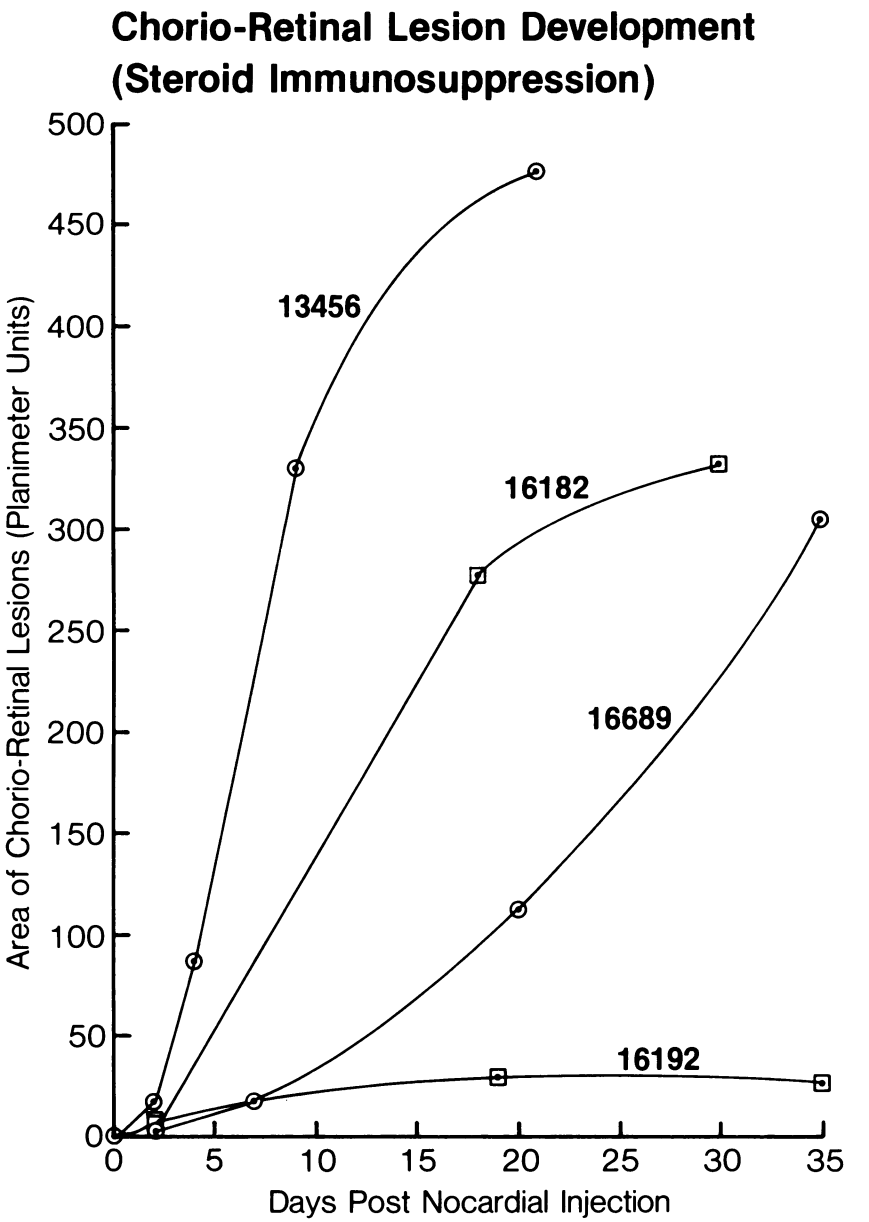

FIGURE 28

Chorioretinal lesion development in rabbits immunosuppressed with steroids alone and infected with *N asteroides* GUH-2. Rabbit No 13456 received 6-methylprednisolone, 30 mg/kg. Rabbit No 16182 received 6-methylprednisolone, 2.5 mg/kg. Rabbit No 16689 received 6-methylprednisolone, 30 mg/kg, and sulfadimethoxine therapy (regimen 3). Rabbit No 16192 is a nonimmunosuppressed, infected control.

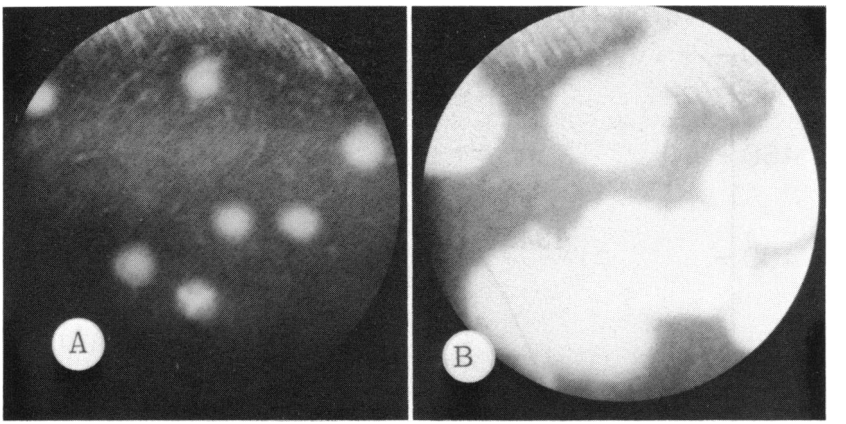

FIGURE 29
A: Retina of rabbit No 14926 (immunosuppressed with 6-methylprednisolone, 30 mg/kg, and cyclophosphamide, 100 mg/kg), 2 days after bacterial challenge. Multiple lesions are seen. B: Same lesions at 6 days. Marked enlargement is noted. Rabbit died 10 days after infection.

organisms while he was shoveling moist hay in a confined space. The *Nocardia* was cultured from the prostate and eye in the first case; from a transtracheal aspirate, sputum, and blood in the second patient; and from the eye, brain, epididymis, lung, and kidneys in the third case. The first two patients exhibited decreased vision, while the last patient complained of pain, redness, and a foreign body sensation in his eye. No right or left eye predisposition was noted: the involvement was equally distributed. It

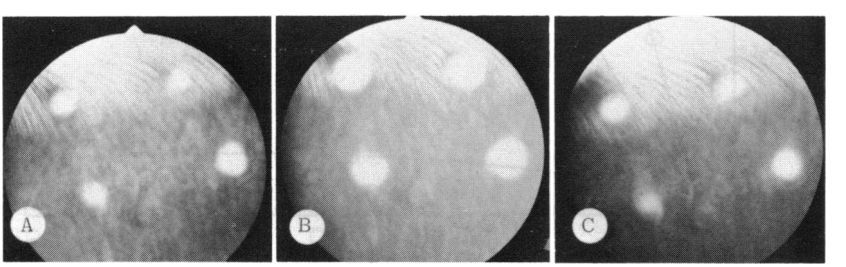

FIGURE 30
A: Retina of rabbit No 14927 (immunosuppressed with 6-methylprednisolone, 30 mg/kg, and cyclophosphamide, 100 mg/kg), 2 days after bacterial challenge. Four lesions are seen. Rabbit was being treated with IM ampicillin and sulfisoxazole diolamine (regimen 4). B: Same lesion 6 days after infection. C: Same lesion 10 days after infection. Rabbit died 11 days after nocardial challenge, but growth of lesions was markedly inhibited by antimicrobial agents.

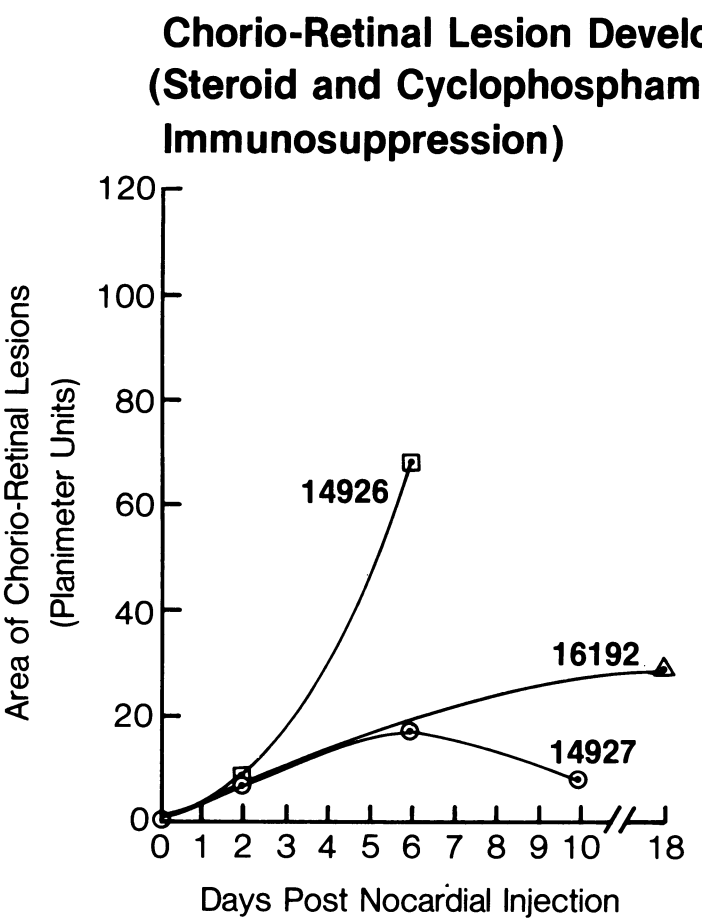

FIGURE 31

Development of chorioretinal lesion in rabbits immunosuppressed with steroids and cyclophosphamide and infected with *N asteroides* GUH-2. Rabbit No 14926 received 6-methylprednisolone, 30 mg/kg, and cyclophosphamide, 100 mg/kg. Rabbit No 14927 received same immunosuppressive medications and ampicillin-sulfisoxazole therapy (regimen 4). Rabbit No 16192 is a nonimmunosuppressed, infected control.

should be noted that one of the three patients had bilateral involvement (case 2). The clinical ocular findings included chorioretinal exudative lesions with hemorrhage in cases 1 and 2 and an anterior uveitis and endophthalmitis in case 3. The first patient was treated with sulfadiazine, sulfisoxazole, ampicillin, and streptomycin, and the second patient was treated with trimethoprim-sulfamethoxazole. The third patient was not

treated because the diagnosis was not made until autopsy. The first patient died, presumably as a result of early discontinuation of the sulfisoxazole, a probable allergic skin reaction to this medication, and the increase in corticosteroid therapy following a renal-transplant-rejection reaction. The second patient apparently survived because of the early diagnosis, the institution of effective antimicrobial therapy (sulfisoxazole-trimethoprim), and the rapid tapering of the prednisone therapy.

The ocular histopathologic findings included lymphocytic and polymorphonuclear leukocytic infiltration in the choroid in case 1 and diffuse nongranulomatous anterior and posterior uveitis and vitreoretinitis in case 3. In both instances, typical organisms were seen in the tissues. No ocular specimen was available in case 2. Two of the three patients, one of whom was immunosuppressed, died as a result of the nocardiosis.

An animal model for disseminated nocardiosis was produced by the injection of 10^3 CFU of *N asteroides* GUH-2 into the carotid artery or marginal ear vein of immunosuppressed Dutch-Belted rabbits. A disseminated disease was produced in steroid-immunosuppressed rabbits challenged with intravascular *Nocardia*. The intra-arterial model allowed the investigator to follow the progression of the disease in a living animal by observing lesions in the retina of the eye. These lesions occurred in 47 of the 76 animals (62%) that were given the bacterial challenge in the carotid artery. If eye lesions developed, they were always present in the ipsilateral eye, and in 4 of the 76 animals (5.3%), lesions also developed in the contralateral eye. Iris lesions occurred in 6.6% of the intra-arterially injected rabbits and in 1.6% of the IV injected rabbits. In humans, lesions in the eye have been reported in about 3% of the cases of disseminated disease.[25] This observation is confirmed in the present experimental study. The same information can also be deduced by assuming that the frequency of eye lesions contralateral to the carotid artery injection would be approximately the same as the frequency following IV injection or dissemination. Within the extensive study group of rabbits, a crossover infection (ie, contralateral eye infection) was observed in only four animals (4 of 76 = 5.3%), thus indicating a low rate of dissemination to the eye. Of those patients with ocular involvement reviewed and reported in this study, however, 31% (4 of 13) had bilateral ocular nocardiosis.

Immunosuppression by long-acting steroid treatment alone allowed the production of progressive disseminated disease following bacterial challenge. These results agree with those of Mishra et al,[98] who produced nocardiosis in mice after repeated cortisone treatment. The 30-mg/kg dose of 6-methylprednisolone in rabbits has been shown by Nolan et al[140] to decrease granulocyte adherence and to cause a shift of granulocytes to

the circulating pool; however, no changes in chemotaxis, phagocytosis, or intracellular killing of organisms have been observed by these investigators.

The combination of cyclophosphamide and steroid pretreatment, intended to duplicate the immunosuppressive regimen given to human renal transplant patients, was successful in further reducing the rabbit host defenses to allow a nonlethal dose of *Nocardia* to produce a fatal disease.

The specific mechanisms of host immunity to nocardial infection have not been established completely. *Nocardia asteroides* has been shown to survive intracellularly and has been considered a facultative intracellular parasite.[36] Cell-mediated immunity should, therefore, play a role in host defense. Circulating antibodies and delayed hypersensitivity to nocardial antigens have been noted in humans and animals, but their roles in normal host defense against nocardial infection are not known.[36,130] *Nocardia* organisms are readily phagocytized by both polymorphonuclear leukocytes and macrophages.[42,47,54,58,141] For the body to eliminate *Nocardia*, cell-mediated mechanisms must be intact. In the present study, the nonimmunosuppressed animals injected with 10^3 CFU of *N asteroides* GUH-2 did not succumb to the infection. Beaman and Maslan[97] demonstrated the difference between steroid- and cyclophosphamide-induced disease. Results with GUH-2 indicate that cyclophosphamide preferentially affects B cells (humoral immunity), while steroids affect T-cell function (cell-mediated immunity). In the present study, treatment of rabbits with cyclophosphamide alone did not increase mortality following nocardial challenge ($P = 0.4$), but when combined with a steroid, the agents appeared to act synergistically, and the mortality was significantly greater ($P = 0.0001$). Cyclophosphamide immunosuppression was not associated with retinal lesions in any of the four rabbits injected intra-arterially, while 83% of saline-treated rabbits and 62.5% of the steroid-treated rabbits had retinal lesions. This may have been due to actual stimulation of cell-mediated immunity by cyclophosphamide through inactivation of the suppressor T cells.[86] Steroids stabilize lysosomal granules, thereby decreasing the intracellular killing ability of the macrophages. Those animals that were treated with a steroid such as 6-methylprednisolone had a mortality related to increasing dosages of the agent. The animal model produced in the present study could be used to further identify the role of these specific host factors.

The results of the antimicrobial treatment studies affirm the serious nature of nocardiosis in immunosuppressed animals in spite of antimicrobial therapy. All seven nonimmunosuppressed animals survived the in-

fection with only minimally scarred lesions in organs including the eye, lungs, and kidneys. Without the nocardial challenge, immunosuppression with steroids was well tolerated. The 16 animals immunosuppressed with steroids (30 mg/kg) and challenged with *N asteroides* died with widespread lesions in virtually every organ system. Oral ampicillin was ineffective in resolving the infection, as determined by the failure to reduce mortality, prolong life, or reduce the size or extent of the widespread nocardial lesions (regimen 1). Serum drug levels were not measured in these animals, and there is reason to believe that this dose of ampicillin may have yielded blood levels below the MIC. This observation is based on a deduction from a similar treatment (by the manufacturer) in humans that gave a blood level of from 2.5 μg/ml to 4.0 μg/ml from a dosage of 28.6 mg/kg/day, which is below the determined MIC for ampicillin (40 μg/ml) when tested with *N asteroides* GUH-2 (Table V).

The trimethoprim-sulfamethoxazole combination therapy (regimen 2) was also ineffective when used in 6-methylprednisolone-immunosuppressed infected rabbits. The manufacturer indicates that a dosage of 6.9 mg/kg/day will yield blood levels of 53 μg/ml of sulfamethoxazole. This level is considerably higher than the determined MIC for sulfa (Table V). Thus, the initial dosage of 40 mg/kg/day should have been more than adequate. The manufacturer's recommended dosage is 48 mg/kg/day, or slightly more than the dosage given to the rabbits. Thus, the level of oral sulfamethoxazole, theoretically, was a reasonable dosage to treat the infection. The lack of a therapeutic response could be attributed to steroid inhibition of cell-mediated immunity or to an added toxic effect of the antibiotics, as in Herxheimer's reaction. A bacteriostatic drug (such as sulfamethoxazole) does require intact cellular defenses to be effective.

The trimethoprim-sulfamethoxazole seemed effective, reducing mortality from 50% to only 12% in those rabbits immunosuppressed with the short-acting methylprednisolone sodium succinate, but the differences were not statistically significant ($P = 0.1$).

Treatment with oral sulfadimethoxine (regimen 3) did protect 50% of the 6-methylprednisolone-treated animals. The animals that did die, died at times similar to those of control animals. Necropsies on these animals showed smaller lesions than were found in control animals. Animals that lived past the 28-day observation period and that were killed later by CO_2 inhalation had small focal lesions in various organs, especially the lungs and kidneys. The recommended adult dosage of sulfadiazine (similar to sulfadimethoxine) is 14 mg/kg/day. This treatment regimen given to rabbits was determined to be partially effective by the 50% survival in this group ($P = 0.003$). Sulfadimethoxine therapy in rabbits immunosup-

pressed with the short-acting steroids, however, did not lower the mortality.

An antimicrobial effect was noted ophthalmoscopically in the sulfadimethoxine-treated 6-methylprednisolone-immunosuppressed animals when lesion growth rate was measured (Fig 29, No 16689). The shift of the growth curve to the right indicates a slower lesion growth rate that could be attributable to the antimicrobial treatment.

Treatment with a combination of IM ampicillin and sulfisoxazole diolamine (regimen 4) did not afford protection from the infection in terms of reduced mortality, but the lesions were much smaller than lesions observed in the retinas (Figs 30 and 31, No 14927) or in the lungs and kidneys (Fig 15) of nontreated animals.

In humans, an ampicillin dosage of 57 mg/kg/day will yield blood levels of up to 6 μg/ml. This is below the MIC (40 μg/ml), as determined in the present study (Table V). The rabbit dosage in regimen 4 was actually about three times greater (170 mg/kg/day vs 57 mg/kg/day), so that the level of ampicillin in regimen 4 should have been adequate, as indicated by retinal lesion regression in those rabbits receiving this combination of antimicrobial treatment. The recommended adult dosage of sulfisoxazole diolamine is 100 mg/kg/day. The dosage of 800 mg/kg/day for 3 days followed by 200 mg/kg/day may have been excessive. Deaths of these animals may have been drug-related.

The present study demonstrates the marked importance of immunosuppressive therapy: a fatal disseminated disease has been produced in immunosuppressed rabbits challenged with a dose of *N asteroides* that was not lethal in nonimmunosuppressed rabbits. Table IX summarizes groups with respect to the influence of roughly equivalent dosages of pretreatment steroids and also shows the influence of continued dosage of the short-acting steroids. The saline pretreatment was associated with no mortality, while treatment with 6-methylprednisolone at a dose of 30 mg/kg caused a 100% mortality ($P < 10^{-6}$). When a comparable dosage of short-acting steroid was given (over 3 days), the mortality was only 10%. The *P* value (when this treatment was compared with the saline pretreatment) was 0.39, indicating no significant difference. If the dose of 10 mg/kg of methylprednisolone sodium succinate was continued daily after infection, however, the mortality was 50% ($P = 0.026$, compared with the saline group [C]). When the 6-methylprednisolone group (D) was compared with the methylprednisolone sodium succinate group (Q) in which the total preinfection steroid dosages were equivalent (30 mg/kg at one dose, compared with three daily doses of 10 mg/kg), the *P* value was less than 10^{-6}. When group D was compared with group S, the *P* value was

TABLE IX: EFFECT OF STEROID PRETREATMENT FORM AND POSTINFECTION ADMINISTRATION ON RABBIT MORTALITY FOLLOWING INTRAVASCULAR CHALLENGE WITH 10^3 CFU *NOCARDIA ASTEROIDES* GUH-2

GROUP	PRETREATMENT MEDICATION	TOTAL PRE-INFECTION DOSAGE	CONTINUOUS DAILY DOSAGE AFTER INFECTION	NO RABBITS	ROUTE OF BACTERIAL CHALLENGE*	MORTALITY RATE (%)†
C	Saline	. . .	. . .	7	IA	0
D	6-Methylprednisolone	30 mg/kg	. . .	16	IA	100
Q	Methylprednisolone sodium succinate	30 mg/kg	. . .	10	IV	10
S	Methylprednisolone sodium succinate	30 mg/kg	10 mg/kg	10	IV	50

*IA = intra-arterial; IV = intravenous.
†At 28 days.

0.0017. This shows the markedly different effects of intermittent steroid medication and treatment with a long-acting preparation. Comparing group Q with group S demonstrated the importance of discontinuation of steroids. This is important clinically when treating an immunosuppressed patient in whom nocardiosis develops. Group Q is comparable to a group of patients in whom the steroids would be discontinued as soon as the diagnosis has been made, while in group S, the steroids were continued. Discontinuation of steroids was associated with a 10% mortality, while the continued use of steroids was associated with a 50% mortality; the difference is significant ($P = 0.05$).

In spite of antimicrobial therapy, the mortality in animals immunosuppressed with long-term steroids was extremely high (23 of 26 = 88%), while in animals immunosuppressed with continuous short-acting steroids and treated with antimicrobial agents, the mortality was only 31% (5 of 16) ($P = 0.001$). These results agree with studies in humans in which antimicrobially treated immunosuppressed patients have been reported to have an 80% to 100% mortality, while nonimmunosuppressed patients have only a 15% to 20% mortality.[33] These findings indicate that in the management of nocardiosis in the immunocompromised patient, immunosuppressive therapy should be decreased to the lowest possible level to allow the maximum T-cell and macrophage function. The rabbit model described in this study could be used for further research to elucidate the T-cell relationship in host defense. Studies of antimicrobial therapy should include measurements of serum levels to correlate in vitro and in vivo sensitivity tests.

One of the advantages of this experimental model is that it allows the investigator to follow the development of the lesions in the retina and to observe development or regression of the lesions with immunosuppressive therapy or antimicrobial treatment.

CONCLUSIONS

Based on the previous clinical and histopathologic study, the following conclusions were made:

1. Disseminated nocardiosis can occur in immunologically intact, immunocompromised, or immunosuppressed patients.

2. Immunologically intact patients may require a larger pulmonary inoculation of organisms than those who are immunodeficient. Once the infection becomes established in a previously immunocompetent patient, however, the organisms themselves can produce immunosuppression by inhibiting phagosome-lysosome fusion.

3. The probability of surviving nocardiosis is increased by rapid diagnosis, discontinuation or marked reduction of immunosuppressive medication, and prolonged, aggressive, and accurate antimicrobial therapy.

4. A reproducible animal model for disseminated nocardiosis was produced in Dutch-Belted rabbits by intravascular challenge with the organism.

5. A reproducible model for endogenous ocular nocardiosis was produced in these rabbits by the intracarotid artery injection of *N asteroides*, producing iris, eyelid, and chorioretinal lesions.

6. A nonlethal infection was produced by the intravascular injection of 10^3 CFU *N asteroides* GUH-2 in nonimmunosuppressed rabbits.

7. Immunosuppression with increasing doses of steroids was associated with increasing mortality in these rabbits.

8. Combined immunosuppression (steroids and cyclophosphamide) was more lethal than when cyclophosphamide or steroids alone were used.

9. Long-acting steroid immunosuppression was more lethal than an equivalent dose of a short-acting preparation.

10. Continuous steroid immunosuppression was more lethal than discontinuing the steroids.

11. A lack of correlation between the in vivo and in vitro drug sensitivity testing was noted. With immunosuppression, drugs active in vitro do not necessarily lower mortality when used in vivo.

12. The mortality in these rabbits in the experimental model was progressively lowered from 100% to 0% in a series of steps.

(*a*) 100% mortality in rabbits immunosuppressed with long-term steroids without antimicrobial therapy (group D).

(*b*) 100% mortality in rabbits immunosuppressed with long-term steroids with ineffective antimicrobial therapy (group U).

(*c*) 50% mortality in rabbits immunosuppressed with long-term steroids and effective antimicrobial therapy (group V).

(*d*) 50% mortality in rabbits immunosuppressed with continuous short-term steroids, without antimicrobial therapy (group S).

(*e*) 50% mortality in rabbits immunosuppressed with short-term steroids, receiving ineffective antimicrobial therapy (group Z).

(*f*) 12% mortality in rabbits immunosuppressed with continuous short-term steroids, receiving effective antimicrobial therapy (group Y).

(*g*) 10% mortality in rabbits initially immunosuppressed with short-term steroids that were then discontinued (group Q) after infection.

(*h*) 0% mortality in nonimmunosuppressed rabbits.

13. Chorioretinal lesions were much more frequent (62%) than iris

lesions (6.6%) in rabbits receiving the *Nocardia* by a carotid artery injection.

14. Iris lesions were more frequent when the *Nocardia* was injected intra-arterially (6.6%) than when injected intravenously (1.6%).

15. Contralateral ocular involvement (5.3%) was less frequent than ipsilateral ocular involvement (62%) when the *Nocardia* was injected in the carotid artery.

16. Contralateral ocular involvement (5.3%) was comparable to the previously reported ocular dissemination rate in humans (3%).

17. Ocular lesions developed in none of the cyclophosphamide-pretreated animals. This may be attributable to stimulation of cell-mediated immunity by cyclophosphamide's adverse effect on suppressor T cells.

18. Early chorioretinal lesions in immunosuppressed rabbits resemble *Staphylococcus* colonies on a blood agar plate or acute argon laser lesions.

19. The later chorioretinal lesions can resemble irregular cryosurgical lesions.

20. Low doses of long-acting steroids facilitated chorioretinal lesion development and retinal detachment without death; when the effect of the steroid passed, the lesions healed.

21. Short-acting steroid immunosuppression was associated with a slower development of chorioretinal lesions.

22. Retinal lesion growth rate can be quantitated using a compensating polar planimeter. The effects of steroid dose and antimicrobial therapy can be measured in terms of progression or regression of the chorioretinal lesions.

23. *Nocardia asteroides* can withstand freezing for at least 12 months without an alteration in its microbiologic, morphologic, or biochemical properties and can withstand freezing for at least 24 months without an alteration in its microbiologic or morphologic properties.

APPENDIX

A number of microbiologic, morphologic, and biochemical properties of the *N asteroides* GUH-2 were determined. The organism, as originally obtained, was compared with the organism that, after 1 year of freezing, was injected intravascularly and then recovered from a renal abscess in a rabbit.

Tables X and XI list the tests performed on each of the nocardial samples and show that all three of the samples (the one originally obtained, the one being used after 12 months of freezing, and the one obtained from the kidney abscess of an animal which died of disseminated nocardiosis) were identical when a number of properties were compared.

TABLE X: PROPERTIES OF *NOCARDIA ASTEROIDES* GUH-2 RECOVERED FROM EXPERIMENTALLY INFECTED RABBITS*

PROPERTY	STRAINS INJECTED INTO RABBIT	STRAINS RECOVERED FROM RABBIT KIDNEY	ORIGINAL PARENTAL STRAIN, *N ASTEROIDES* GUH-2
Pigment produced on BHI agar	Beige with soluble brown pigment	Beige with soluble brown pigment	Beige with soluble brown pigment
Acid-fast (Kinyoun's method)	Weak	Weak	Weak
Decomposition of:			
Urea	+ (slow)	+ (slow)	+ (slow)
Casein	−	−	−
Hypoxanthine	−	−	−
Xanthine	−	−	−
Tyrosine	−	−	−
Hydrolysis of:			
Esculin	+	+	+
Starch	−	−	−
Acid from:			
Glucose	+	+	+
Mannose	±	±	±
Mannitol	−	−	−
Maltose	−	−	−
Arabinose	−	−	−
Rhamnose	−	−	−
Sorbitol	−	−	−
Sucrose	−	−	−
Galactose	−	−	−

*Table X is reproduced courtesy of Blaine L. Beaman, PhD.

TABLE XI: PROPERTIES OF *NOCARDIA ASTEROIDES* GUH-2 RECOVERED FROM EXPERIMENTALLY INFECTED RABBITS*

PROPERTY	STRAINS INJECTED INTO RABBIT	STRAINS RECOVERED FROM RABBIT KIDNEY	ORIGINAL PARENTAL STRAIN, *N ASTEROIDES* GUH-2
Utilization of:			
Acetamide	−	−	−
Proprionate	+	+	+
Valine	+	+	+
Isoamyl alcohol	−	−	−
2,3-Butylene glycol	+ (slow)	+ (slow)	+ (slow)
Citrate	−	−	−
Ethanol	−	−	−
Gluconate	+	+	+
1,2-Propylene glycol	−	−	−
Quinic acid	−	−	−
Inositol	−	−	−
Size of mycolic acids	C_{44}-C_{58}	C_{44}-C_{58}	C_{44}-C_{58}
Presence of tuberculosteric acid	+	+	+
Profile of fatty acids characteristic of *N asteroides* GUH-2	+	+	+

*Table XI is reproduced courtesy of Blaine L. Beaman, PhD.

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