

ACTINOMYCES VISCOSUS,
A POTENTIAL PATHOGEN IN DOGS

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ABSTRACT

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By

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In one and one-half years, five cases of *Actinomyces viscosus* infections in dogs have been identified at the Michigan State University Veterinary Clinic. Infections were similar to those diagnosed of nocardiosis and actinomycosis during a period of nine years at the Clinic. The possibility of mistaken identification of the etiologic agent in many of these infections should be considered. It seems likely that many were actually *A. viscosus*. *Actinomyces viscosus* is a member of the normal flora in periodontal plaque and has been recently reported in occasional suppurative, granulomatous infections in dogs and humans.

A survey of the history and characteristics of infections with special reference to granule morphology, cellular morphology, and biochemical properties of the strains from these cases is discussed. It was concluded that seven biochemical tests and an acid-fast stain should speciate *A. viscosus* from other diphtheroidal or filamentous microorganisms. The tests are catalase production, urease production, gelatin hydrolysis, Voges-Proskauer, lactose fermentation, aesculin hydrolysis, and nitrate reduction.

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW.	2
Taxonomical Methods	2
Numerical Taxonomy	2
General Characteristics.	4
Cell Wall Composition.	5
Serological Properties	7
Fermentation Studies.	8
Ultrastructure.	11
Isolation of <i>A. viscosus</i>	11
Pathology of Actinomycetales.	21
Confusion of <i>A. viscosus</i> with Other Organisms	28
Treatment of Canine Actinomycosis and Nocardiosis	37
CLINICAL HISTORIES OF CANINE ACTINOMYCOSIS SEEN AT THE MICHIGAN STATE UNIVERSITY VETERINARY CLINIC JUNE 1972 TO NOVEMBER 1973	39
Clinic Case 129820 (CF-470-72).	39
Clinic Case 138631 (CF-99-72)	40
Clinic Case 141564 (CF-295-73).	42
Clinic Case 131568 (CF-492-73).	44
Clinic Case 145833 (CF-1078-73, CF-1097-73)	46
MATERIALS AND METHODS.	50
Organisms	50
Culture Media	50

	Page
Isolation Methods	51
Preservation of Stock Cultures.	51
Morphologic Studies	52
Biochemical Tests	52
Carbon Dioxide Requirements	54
RESULTS.	55
Morphological Characteristics	55
Biochemical Tests	58
DISCUSSION	62
ADDENDUM	69
Clinic Case 147495 (CF-194-74).	69
BIBLIOGRAPHY	71
APPENDIX	77
A. TECHNIQUES FOR ISOLATION AND IDENTIFICATION OF <i>A. VISCOSUS</i> IN THE CLINICAL LABORATORY.	77

LIST OF TABLES

Table		Page
1	Canine actinomycosis: reported characteristics in 8 dogs by Swerczek <i>et al.</i> , 1968	16
2	Canine and feline cases from which filamentous microorganisms were isolated in the Clinical Microbiology Laboratory, Veterinary Clinic, Michigan State University, from 1965 to 1974	29
3	Comparison of biochemical studies of <i>A. viscosus</i> strains isolated at MSU with reported reactions of <i>A. viscosus</i> and <i>A. naeslundii</i> cited in literature	59
A-1	Expected reactions of various Gram-positive filamentous or diphtheroidal microorganisms	79

LIST OF FIGURES

Figure		Page
1	<i>A. bovis</i> sulfur granules with clubbed border.	23
2	Higher magnification of <i>A. bovis</i> sulfur granules.	23
3	CF-1097-73. Histopathology of <i>A. viscosus</i> granules from lung tissue.	48
4a	CF-99-72. Reversion from rough to smooth colonial morphology.	56
4b	CF-99-72. Reversion from rough to smooth colonial morphology.	56
5	CF-194-74. Diphtheroid form of <i>A. viscosus</i> associ- ated with smooth colonial morphology.	57
6a,b	CF-470-72. Filamentous form of <i>A. viscosus</i> associ- ated with rough colonial morphology	57
7	CF-194-74. Histopathology of <i>A. viscosus</i> granules from cervical abscess	70

INTRODUCTION

The seventh edition of Bergey's manual (Breed *et al.*, 1957) places Gram-positive pleomorphic organisms into the families Actinomycetaceae, Corynebacteriaceae, Streptomycetaceae, and Mycobacteriaceae. Many of these organisms, when isolated from human and animal sources, are considered to be normal flora or contaminants. Since these are classified as nonpathogens, cultural identification of these slow-growing, fastidious organisms is not attempted in most clinical laboratories. The organisms are ignored or reported as diphtheroids (Gerencser *et al.*, 1969).

Recently, certain diphtheroids have been isolated in material obtained from cases of periodontal disease, suppurative granulomatous abscesses and empyemas (Coleman *et al.*, 1969; Johnson *et al.*, 1970; Georg *et al.*, 1972; Adeniyi-Jones *et al.*, 1973). The possible pathological significance of these organisms has necessitated better methods of cultural isolation and identification.

During a one and one-half year period, *Actinomyces viscosus* has been isolated from suppurative lesions in five dogs admitted to the Michigan State University Veterinary Clinic. The case histories and cultural studies reported in this thesis should aid the clinician and the microbiologist in the diagnosis of *A. viscosus* infections.

LITERATURE REVIEW

Taxonomical Methods

Numerical Taxonomy

There are two different systems of taxonomy in the analysis used for microorganisms: the Adansonian and the Darwinian. Concern with the validity of the results, time involved, and financial practicality of a computer in the microbiology laboratory has eliminated the Adansonian method from most clinical laboratories. Routine analysis presently relies on monothetic Darwinian principles of taxonomy. No one perfect method for classification of the kingdom Procaryotae has been found, and strict adherence to subclassification levels, similar to those used in the plant and animal kingdoms, is almost impossible. The eighth edition of Bergey's *Manual of Determinative Bacteriology* (to be printed in May 1974) proposes the taxonomic structure of 19 parts, many of these lacking class, order, or family (Langlykke, 1973).

Harrington (1966) used numerical taxonomy to study diphtheroids included in the genera *Mycobacteria*, *Corynebacterium*, and *Nocardia*. Morphological, metabolic and cell wall studies led to the conclusion that consistent data were lacking to justify use of three separate genera. The seventh edition of Bergey's manual uses acid-fastness and cellular morphology as criteria for genera separation; however, some

species of *Nocardia* have been shown to be non-acid-fast, and some *Corynebacterium* sp. show varying degrees of acid-fastness (Harrington, 1966). Bousfield *et al.* (1969) also attempted classification of coryneform bacteria with numerical taxonomy. The group of coryneforms included *Microbacterium*, *Cellulomonas*, *Listeria*, *Corynebacterium*, *Brevibacterium*, *Nocardia*, *Mycobacterium*, *Erysipelothrix*, and *Arthrobacter*. He also had little success in genus separation. Holmberg and Hallander (1972b) successfully used a computer in their numerical taxonomic methods to classify 123 diphtheroid isolates from human oral, pathological, and typed reference material. The 77 characteristics examined formed phenon clusters of the genera *Corynebacterium*, *Bacterionema*, *Rothia*, *Actinomyces*, and *Nocardia* with a similarity level of 87.5%. *Actinomyces viscosus* and *A. naeslundii* agreed on a level of 92.5% and thus did not warrant separate species names. Analysis of data involved use of the similarity coefficient S_j (Sneath, 1957a), simple matching coefficient S_s (Sokal *et al.*, 1958) and single cluster analysis dendrogram (Sneath, 1957b). About 50% of the characteristics examined were morphological or colonial. Diphtheroids can demonstrate variations in physical and colonial morphology which leaves much doubt as to the significance of taxonomic data based on such characteristics (Gordon, 1966).

Even though *A. viscosus* and *A. naeslundii* may be found in similar specimens and have many characteristics in common, other taxonomic methods have demonstrated definite differences between these organisms. Cell wall composition, serology, and a limited number of metabolic

properties demonstrate consistent differences sufficient to justify the present use of two separate species names.

The eighth edition of Bergey's manual (Langlykke, 1973) separates coryneform organisms into one part, viz., 17: "Actinomycetes and Related Organisms." *Erysipelothrix*, *Listeria* and Lactobacillaceae are not considered in this part. *Corynebacterium* is grouped without an order or family. The order Actinomycetales includes the families Actinomycetaceae, Mycobacteriaceae, Actinoplanaceae, Frankiaceae, Dermatophilaceae, Nocardiaceae, Streptomycetaceae, and Micromonosporaceae. The family Actinomycetaceae includes the genera *Actinomyces*, *Arachnia*, *Bifidobacterium*, *Rothia*, and *Bacterionema*. The criteria on which this classification is based are not available at this time, as the manual has not yet been published (Langlykke, 1973).

General Characteristics

The family Actinomycetaceae defined by cell wall composition and cellular morphology cannot be placed in the present classes of Schizomycetes or Mycota (Sykes *et al.*, 1973; Prevot, 1966). Organisms are non-acid-fast, Gram-positive, nonmotile, and possess a transitory branching mycelial phase. No true spores or aerial mycelia are present. The cells are about 1.0 μ in diameter and occasionally break into coccoidal or rod-like elements. Cell membranes lack sterols and cell walls lack cellulose, chitin, and diaminopimelic acid (except *Arachnia*). Antibacterial agents are toxic to cells. The absence of sterols in cell membranes makes the organisms insensitive to polyene antifungal agents (Davis *et al.*, 1973). Phage infection and lysozyme susceptibility have been reported in some species (Gledhill *et al.*, 1969).

Membranous organelles are not present. Products of glucose metabolism include acetic, formic, succinic and lactic acids, and some have traces of propionic acid (Li *et al.*, 1968). Catalase production varies and addition of carbon dioxide necessary for anaerobic growth usually enhances aerobic growth in facultative species. Oxygen requirements vary (Gledhill *et al.*, 1969). No gas is produced from sugar fermentation. Hydrolysis of gelatin and production of indol are not seen. Cells may form long branched filaments or pleomorphic diphtheroidal forms, both with round swollen primitive spores or clubbed ends.

Cell Wall Composition

Cell wall composition has become an important tool in taxonomy of the diphtheroids. Actinomycetes lack diaminopimelic acid (DAP) which is characteristically found in its meso form in *Nocardia*, *Mycobacteria*, *Bacterionema*, and some *Corynebacterium* species. Cell walls of fungi rarely contain DAP or amino acids (Cummins *et al.*, 1958; Cummins, 1961). Boone and Pine (1968) partially identified many species of the Actinomycetales by cell wall composition. Complete speciation would have involved quantitative measurements of the cell wall components. Diphtheroids containing LL-DAP are *Corynebacterium acnes*, *Streptomyces* sp. and *Arachnia*. *Bifidobacterium*, *Actinomyces*, *Rothia*, *Lactobacillus*, and some corynebacteria lack DAP. Lysine is present in the above species of corynebacteria, *Rothia*, *Lactobacillus* and *Actinomyces*. Actinomycetes also contain mannose and ornithine while one or both of these are missing in the cell walls of *Lactobacillus* and *Rothia*. Glycine is found in some corynebacteria but not in actinomycetes.

Only *C. pyogenes* cannot be differentiated from *Actinomyces* sp. by these techniques (Pine, 1970; Cummins, 1961).

Reed (1972) examined cell wall components of Strain T-6, *A. viscosus*, with the gas chromatograph. The only amino sugars isolated were N-acetyl-glucosamine, and N-acetyl muramic acid, both found in a peptidoglycan. Rhamnose was the major neutral sugar. No sugar alcohol or techoic acids were present. Phosphorus and phospholipids were in small amounts and amino acids detected included alanine, lysine, and glutamate. Other studies (Pine *et al.*, 1965; Pine, 1970; Gerencser *et al.*, 1969) found galactose, glucose, mannose, and ornithine. Boone and Pine (1968) detected trace amounts of phenylalanine, methionine, leucine, and isoleucine. Cell walls of *A. naeslundii* contain fucose and deoxytalose which have not been found in *A. viscosus* (Pine, 1970).

Two colonial morphologies of actinomycetes are commonly noticed: a spidery, granular, irregular form, and a smooth, entire, convex form (Ajello *et al.*, 1963). A difference in cell wall composition may be responsible for the morphological variations (Sykes *et al.*, 1973; Pine *et al.*, 1967). Moderate quantities of hexosamines, and little or no aspartate, are present in spidery colonies, while low amounts of hexosamines and larger quantities of aspartate are in the smooth colonies. Pine (1970) admits that morphological changes may be associated with variation in cell wall composition; however, changes in the site of cell wall synthesis or enzymatic differences may be responsible for morphological variation. Neither smooth nor rough forms of *A. viscosus* contain aspartate in the cell walls.

Serological Properties

Serological reactions of *A. viscosus* and other diphtheroids have been examined with agglutination, immunodiffusion, and fluorescent antibody techniques. Agglutination studies produced many cross reactions. Snyder *et al.* (1967a) found strains of *A. viscosus* agglutinated with antisera prepared from *A. israelii*, *A. naeslundii*, *A. odontolyticus*, *R. dentocariosa*, and *B. matruchotii*. The antisera from *C. acnes* and *A. odontolyticus* reacted with *A. viscosus* in studies by Slack *et al.* (1971). Immunodiffusion gel studies (Snyder *et al.*, 1967a) with *A. viscosus* cells showed minor cross reactions with *A. bovis*, *A. odontolyticus*, and *A. naeslundii* antisera.

An excellent serological test for *A. viscosus* is the fluorescent antibody technique. Hamster strains of *A. viscosus* do not react with antisera from human *A. viscosus* strains or with *A. naeslundii* antisera (Holmberg *et al.*, 1972b; Gerencser *et al.*, 1969). Human strains did react with *A. naeslundii* antisera and with hamster *A. viscosus* antisera. Reciprocal tests of *A. naeslundii* isolates with *A. viscosus* antisera from either hamster or human strains reacted (Gerencser *et al.*, 1969). When *A. viscosus* hamster antisera was sorbed with *A. naeslundii*, all cross reacting antibodies were removed; thus no reaction with human strains of *A. viscosus* or with *A. naeslundii* was possible. Antisera prepared from *A. viscosus* did not fluoresce at diagnostic titers with cultures of *A. israelii*, *A. bovis*, *A. odontolyticus*, *A. eriksonii*, *A. propionica*, *R. dentocariosa*, *B. matruchotii*, *C. pyogenes*, *C. acnes*, or *Ramibacterium pleuriticum* (Gerencser *et al.*, 1969). Studies of fluorescent antibody reactions with *A. viscosus* of human, hamster, and canine strains showed cross reactions of both human

and hamster strains with canine strain antisera (Georg *et al.*, 1972). Types of cross reacting antigens varied with canine strain examined since absorption with *A. naeslundii* had variable effects.

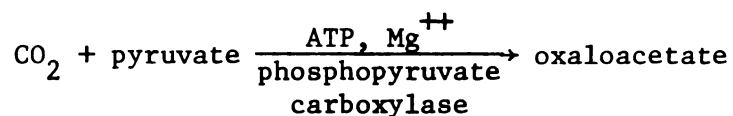
Fermentation Studies

Gas-liquid chromatography of fermentation end-products is not only a useful technique for speciation of anaerobic bacteria (Henis *et al.*, 1966), but it can also provide useful information for gross separation of some anaerobic diphtheroidal organisms (Pine, 1973; Li *et al.*, 1968).

Three types of detection apparatuses have been used for determination of volatile and nonvolatile acids: flame ionization, electron capture, and thermal conductivity. The electron capture device is extremely sensitive for identification of organic compounds with halogenated or polar functional groups (Cherry *et al.*, 1969). Flame ionization is very sensitive to formic acid and less sensitive to water than the thermal conductivity device, but acetic acid carryover may occur when multiple samples are being tested (Moore *et al.*, 1966). Li and Georg (1968) successfully used the flame ionization detector to separate *Ar. propionica* from other actinomycetes. Qualitative and quantitative analysis with this detector differentiated *Actinomyces* from *Corynebacterium*, *Nocardia*, and other diphtheroids (Pine, 1973).

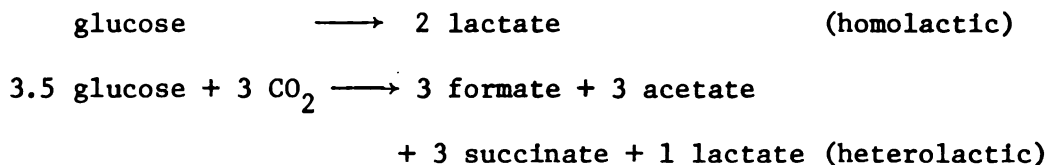
Howell (1963) noted that *A. viscosus* requires carbon dioxide for aerobic growth but *A. naeslundii* does not. Buchanan *et al.* (1963) suggested that actinomycetes lack aspartate permease and thus must synthesize aspartic acid from CO₂ and small molecules. Large amounts

of carbon dioxide are fixed into the carbonyl group of oxaloacetate by the Wood-Werkman reaction:

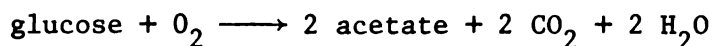


(Wood *et al.*, 1941; Buchanan *et al.*, 1966). Other CO₂ fixation mechanisms are operative but these are not major reactions. The addition of carbon dioxide to a growing culture of an actinomycete will rapidly increase growth and production of several end-products. Proposed equations of glucose fermentation by *A. israelii*, *A. bovis*, *A. viscosus*, and *A. naeslundii* had evolved through extensive chemical extractions and titrations performed before the gas chromatograph became popular for such research (Buchanan *et al.*, 1966; Buchanan, 1967; Pine, 1970).

Anaerobic



Aerobic

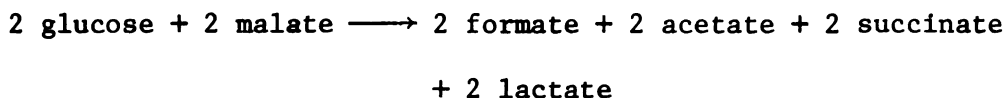


Malate can substitute for carbon dioxide under anaerobic conditions to produce:

low glucose concentration

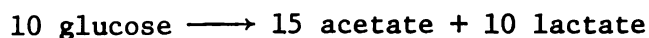
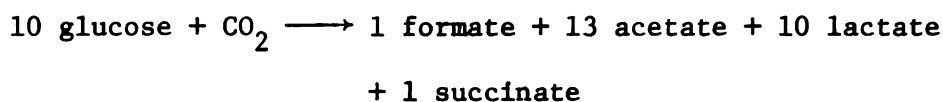


high glucose concentration

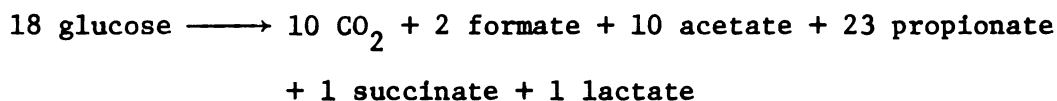


Other diphtheroids do not require large amounts of carbon dioxide for growth. *Arachnia propionica*, *Bifidiobacterium bifidum*, *Propionibacterium*, and *Corynebacterium acnes* have the following fermentation balances (Pine, 1970):

B. bifidum



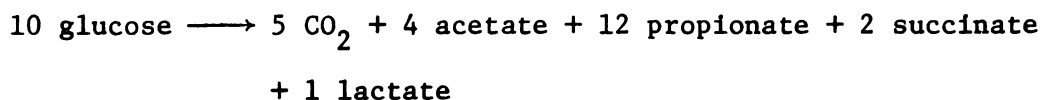
Ar. propionica



P. pentoaceum, *P. arabinosum*



C. acnes



Actinomyces sp. ferment glucose predominantly by the Embden-Meyerhof pathway; however, the organisms also use the hexose monophosphate shunt, phosphoketolase pathway, and Entner-Doudoroff pathway to a slight extent (Pine, 1970). When carbon dioxide is added to anaerobic cultures of *Actinomyces* sp., fermentation changes from homolactic to heterolactic. Carbon dioxide acts as a hydrogen acceptor to oxidize lactate, thus

forming formate and acetate from a pyruvate intermediate. Carbon dioxide is also used to form oxaloacetate from which aspartate can be synthesized. Energy yielding studies by Payne (1970) showed the addition of malate, CO_2 , and $\text{O}_2 + \text{CO}_2$ to anaerobic cultures increase the energy yield of *A. naeslundii* by 22%, 150%, and 200%, respectively. Presently no enzymatic differences between *A. viscosus* and *A. naeslundii* have been suggested to explain why *A. naeslundii* shows no preference for CO_2 when growing in oxygen but *A. viscosus* does (Howell, 1963).

Ultrastructure

Two studies on ultrastructure in the Actinomycetales have shown some possible differences in species. Overman *et al.* (1963) found complex or coiled membranes in *Ar. propionica*, simple coils in *A. naeslundii* and *A. israelii*, and no coils in *A. bovis* or *Bifidobacterium bifidum*. Duda *et al.* (1972) and Overman *et al.* (1963) noted variations in cell wall thickness in different species; however, these findings showed no relationship to atmospheric requirements of the species.

Isolation of *A. viscosus*

The generic name, *Actinomyces*, was first used by Harz in 1877 (Gerencser *et al.*, 1969), who was describing a "concentrically radiating fungus" obtained from tongues of cows suffering from a disease spreading around the jaw bones. Dr. Bollinger, who originally sent the specimens to Harz, lectured and published papers describing this *Actinomyces bovis*, which Harz identified but could not culture. Israel

and Ponfick identified the disease in humans in 1878, but *Actinomyces* was not actually isolated from human material and injected into animals until 1891 by Wolff and Israel (Conant *et al.*, 1971).

An aerobic filamentous actinomycete resembling *Actinomyces viscosus* was first described by Gruter in Argentina in 1932 (Negroni *et al.*, 1932). He cultured *Actinomyces discofoliatus* from pus present in infected lacrimal concretions and suppurative gum lesions of a patient with an infection aggravated by an ill-fitting artificial palate. The cellular and colonial morphology resembled that of *A. viscosus* but the organism described could not hydrolyze starch or reduce nitrates. It did hydrolyze gelatin and produce indol from tryptophan. Catalase production had not been examined. These biochemical tests led the author to the conclusion that this organism was probably not *A. viscosus*. Brion (1939, 1942) isolated an organism from dog and cat lesions which also resembled *A. viscosus*. Georg *et al.* (1972) noted that this organism, *A. baudetii*, was not preserved for future examination, and Brion gave a description of metabolic properties of the organism too incomplete to adequately exclude the possibility that *A. baudetii* might have been an organism other than *A. viscosus*.

Gerencser and Slack (1969) obtained cultures of *A. baudetii* from the Pasteur Institute in Paris and *A. discofoliatus* from P. Negroni in Argentina. Biochemical, morphological, and serological tests of these organisms demonstrated they were both *A. viscosus*. Further investigation showed these were not the original strains described by Gruter and Brion. Cultures which resembled only the strains' morphological

characteristics had been sent to Gerencser under these names.¹

Filamentous microorganisms became an important topic for research in the field of dental medicine. Aerobic, non-acid-fast filaments isolated from human specimens were identified as *Nocardia salivae*² in 1962 (Roth *et al.*, 1962). Numerous other diphtheroids were also found in the mouth, and the possibility that these might cause disease was suspected. Hamsters orally inoculated with a suspension of *Leptotrichia* sp.,³ a filamentous organism found in dental caries, produced infections (Jordan *et al.*, 1964a). Syrian hamsters fed high carbohydrate diets of confectioners sugar acquired periodontal infections (Jordan *et al.*, 1964b; Keyes *et al.*, 1964). The organism isolated from subgingival plaque in the infections was identified as Strain T-6. This strain was orally inoculated into uninfected hamsters and produced carious lesions from which T-6 could again be isolated. Howell and Jordan (Howell, 1963; Howell and Jordan, 1963) examined the morphological, colonial, and physiological characteristics of Strain T-6 and other diphtheroids isolated from hamster dental plaque. Their observations of an aerobic, catalase positive, filamentous "hamster organism" comprised the first detailed description of *A. viscosus*. The hamster organism was compared with *R. dentocariosus*, another catalase positive, aerobic, non-acid-fast actinomycete present in dental plaque. Only the "hamster organism" could be shown to induce periodontal disease. *Rothia* was beaded and

¹Personal communication from M. A. Gerencser.

²*N. salviae* was later called *N. dentocariosus* and is presently *Rothia dentocariosa*.

³*Leptotrichia* is now *Bacterionema matrilotii*.

did not produce long filaments seen in T-6. Howell (1963) mentioned that *A. naeslundii* had similar morphologic and metabolic properties as T-6; however, *A. naeslundii* was catalase negative and did not need additional carbon dioxide for aerobic growth, which T-6 did require. *Nocardia* and *Rothia* also grow well aerobically without CO₂. End-products from glucose fermentation by T-6 included acetic, formic, lactic, and succinic acids. Howell *et al.* (1965) officially named the hamster organism *Odontomyces viscosus*. At the time the basis for exclusion of T-6 from a new proposed family "Nocardiaceae" included lack of aerial hyphae, different cell wall components, inability to produce true spores, different atmospheric requirements, and fermentative ability. Howell maintained that production of catalase was a significant enough difference to justify the separate genus in the Actinomycetaceae family. Fluorescent antibody tests were performed and no cross reaction with *A. israelii*, but weak reactions with *A. naeslundii*, were seen.

Supragingival and subgingival plaque in infected hamsters showed apical migration of epithelium, erosion of cementum, and inflammation of periodontal pockets, later progressing to erosion of the bone (Jordan *et al.*, 1965b). Hamsters could become infected from direct plaque inoculation, close contact with infected rats, or from consuming large amounts of carbohydrates. *Odontomyces viscosus* showed plaque formation (Jordan *et al.*, 1965a). No evidence of soft tissue invasion in any of these infections could be shown. Some hamsters were presented with enlarged cecums after several weeks, but no filamentous organisms were cultured from the cecums.

Melville (1965) isolated pathogenic plaque producing diphtheroids from the human oral cavity and identified these as *A. naeslundii*. He maintained that some of these strains of *A. naeslundii* were capable of producing catalase. This was the first reference of human infection with a possible strain of *A. viscosus*; however, lack of a full description of the organism prevents verification.

An indirect fluorescent antibody technique to demonstrate reactions to *O. viscosus* showed reactions in one-half of the 14 patients examined by Snyder *et al.* (1967b). The ability of this test to identify *O. viscosus* in patients is questionable since cross reactions occurred with four other organisms: *A. israelii*, 14/14; *A. naeslundii*, 8/14; *R. dentocariosa*, 6/14; and *B. matrighotii*, 14/14. No cultures grew *O. viscosus*. Swerczek *et al.* (1968) examined eight cases of canine actinomycosis (Table 1). Seven of these involved large breeds. It was suggested that actinomycosis occurs frequently in dogs older than one year, especially hunting breeds, since they have a great probability of exposure to soil-contaminated trauma. *Actinomyces* sp. do not occur in soil; however, bacteria from soil may be necessary to contaminate the lesion and debilitate the animal so an endogenous actinomycete can colonize in the wound. Lesions of all dogs contained granules that had non-acid-fast filaments in their centers. Gram stains revealed Gram-positive bacteria and filaments. A distinct border without any club formation differentiated the canine granules from loose, irregular granules seen in nocardiosis and clubbed granules of other actinomycoses. Although no cultural studies were performed to speciate the actinomycete, the author feels there is a strong possibility that the etiologic agent

Table 1. Canine actinomycosis: reported characteristics in 8 dogs by Swerczek *et al.*, 1968

Age	Breed	Case	Diagnosis	Characteristics of Microorganisms*	Size of Granules
9 yr	Springer Spaniel	133931	Empyema actinomycosis	Could not culture	Large
5 yr	Boxer	D7534	Salivary cyst actinomycosis	Not cultured	Microscopic
2 yr	Boxer	F8098	Lung abscess actinomycosis	Not cultured	Microscopic
adult	Golden Retriever	F10592	Thoracic empyema actinomycosis	Not cultured	Microscopic
2 yr	Mixed	F10889	Cerebral abscess actinomycosis	Not cultured	Microscopic
6 yr	Boxer	G10824	Flank abscess actinomycosis		Microscopic
1 yr	St. Bernard	H9887	Cervical lesion actinomycosis	Not cultured	Microscopic
7 yr	Dachshund	H1545	Generalized actinomycosis	Grown anaerobically with other mixed Gram-positive bacilli	Microscopic

* All were Gram-positive, non-acid-fast on direct smears.

was *A. viscosus* in some of these cases. This is based on granule type, acid-fast reaction, and incidence of infections of dogs with various *Actinomyces* species.

In 1967 the *Subgroup on Taxonomy of the Microaerophilic Actinomycetes* amended the general description of the genus *Actinomyces* to then include catalase positive organisms (Georg *et al.*, 1969b). It was suggested that *O. viscosus* be changed to *Actinomyces viscosus*. Isolation of *A. viscosus* in human specimens of dental plaque was characterized and found to possess the same morphological and physiological properties as the hamster strains. Short rods in palisade arrangements and long dividing filaments were found in the same human isolates, and several strains which originally formed rough, heaped colonies later reverted to smooth, entire, colonial formations. Georg *et al.* (1969b) also used serological and cell wall analysis to identify these strains. The hamster organisms had the same cell wall components as the human strains but serological differences were apparent.

Further studies on periodontal disease in hamsters and humans resulted in an interesting theory on colonization and growth requirements. Gram-positive organisms appear to be the predominant cultivable flora in the human oral cavity (Holmberg *et al.*, 1972a). There must be some bacteria present in the mouth before colonization and the induction of caries and periodontal disease can be effected. It appears that *Streptococcus sanguis* is present in early plaque formation in humans. As plaque continues to be deposited, Gram-positive rods and filaments colonize, and *St. sanguis* disappears. The new flora consists of *Corynebacterium*, *Actinomyces*, and *Lactobacillus* species. Cultures

of *St. sanguis* can inhibit growth of *Actinomyces*, *Bacterionema*, *Corynebacterium*, *Nocardia* and *Lactobacillus* on the same culture plates (Holmberg *et al.*, 1972a), which suggests a possibility that inhibition of diphtheroidal colonization by *St. sanguis* may occur *in vitro* in the oral cavity. Large amounts of sucrose catabolites which occur in organisms fed a high carbohydrate diet inhibit the growth of *St. sanguis*, and enhance the growth of *A. viscosus*. *Actinomyces viscosus* readily hydrolyzes sucrose and uses the resulting carbon and energy for other synthetic reactions.

T-6, *A. viscosus*, when fed in large amounts to gnotobiotic hamsters and rats, will rarely produce disease symptoms other than carious lesions (Reed *et al.*, 1972). The lesions found in gnotobiotic rats orally infected with *A. viscosus* or *A. naeslundii* are similar (Socransky *et al.*, 1970; Frank *et al.*, 1972). *Actinomyces viscosus* may be more pathogenic than *A. naeslundii* since orally infected rats fed high carbohydrate diets produced more dental lesions from *A. viscosus* than from *A. naeslundii*.

Actinomyces viscosus and *A. naeslundii* are not as pathogenic as the anaerobic actinomycetes. Few cases of noncarious type infections have been reported (Georg *et al.*, 1972; Lewis *et al.*, 1972; Adeniyi-Jones *et al.*, 1973; Holmberg *et al.*, 1972b). Experiments to compare the pathogenicity of *A. viscosus* and *A. naeslundii* by intraperitoneal inoculation of laboratory animals showed slight differences between the two species (Coleman *et al.*, 1969; Georg *et al.*, 1968). Coleman *et al.* (1969) described three experiments of hamster and mouse infections with *A. naeslundii*. Although *A. naeslundii* could be isolated from

lesions in many of the experimental animals, it was apparent that intraperitoneal inoculations with the obligate anaerobic actinomycetes were significantly more severe and more frequent. The severity of lesions in mice produced from single inoculations of several different *Actinomyces* sp. was examined by Georg *et al.* (1968). *Actinomyces naeslundii* induced lesion formation in 104/119 (87%) mice and *A. viscosus* induced lesions in 23/24 (95%) mice.

Actinomyces viscosus is known to be the causal agent of periodontal disease in hamsters, and it also has been isolated from the human oral cavity on numerous occasions (Georg *et al.*, 1972). Georg *et al.* (1972) examined five strains of *A. viscosus* isolated from animal infections and experimentally inoculated these intraperitoneally into mice and newborn puppies. These strains were highly pathogenic to all of the mice producing abscesses on parietal and peritoneal surfaces which were restricted to the abdominal cavity. Most of the lesions involved the liver. *Actinomyces viscosus* produced lesions in only one of the six newborn puppies. The lesions were in the hepatic parenchyma and resembled those found in one of the dogs presented with the original infection. This is the only published case of a successful experimental infection in a large animal.

Holmberg and Hollander (1972b) felt that the differences between *A. viscosus* and *A. naeslundii* were not significant enough to justify use of two separate species names. A calculated median organism was suggested that would include both species. Otherwise, Holmberg *et al.* recommended that *A. viscosus* be considered a variant of *A. naeslundii*. Their data for evaluation by numerical taxonomy methods did not include

cell wall analysis or serology, both of which are presently used to separate the species.

Two cases of pathological involvement of *A. viscosus* in humans have been described. One was a 35-year-old male who suffered with a bilateral pleural effusion (Lewis *et al.*, 1972). No organism was seen in stains of direct smears of aspirated material; however, *Mycobacterium tuberculosis* and *A. viscosus* were cultured from the specimen. The patient had a positive tuberculin skin test and, because he had tuberculosis, the amount of pathology due to *A. viscosus* could not be adequately described. The patient showed no signs of improvement when only antituberculosis drugs were administered, but did improve when bacterial antibiotics were added to the treatment. The other 19-year-old male was presented with a branchial cyst below the left ear (Adeniyi-Jones *et al.*, 1973). The cyst contained hemorrhagic tissue resembling that seen in chronic inflammations and was filled with a yellow, nonodorous pus which was aspirated and stained. The Gram stain showed clumps of Gram-positive pleomorphic diphtheroid-like rods and some short filaments; some organisms had swollen ends or were filamentous. Only *A. viscosus* was grown in culture, and colonies on agar were of the smooth form: entire, glistening, white, convex. This, however, formed granular floccules when cultured in broth. Biochemical tests confirmed the identification of *A. viscosus*, and a positive reaction with the fluorescent antibody test at the Center for Disease Control (CDC) in Georgia was reported later.

Holmberg and Hollander (1972b) stated that "no clear relationship of *A. viscosus* to human infections has been shown." Personal

communication with Dr. L. K. Georg has provided the author with two strains of *A. viscosus* isolated from a human lung biopsy and a lung abscess. Attempts to fulfill Koch's postulates with these strains from human specimens have not been made. It is thus impossible to unequivocally state *A. viscosus* can cause disease in man.

Pathology of Actinomycetales

The term "actinomycosis" usually refers only to *Actinomyces* infections and nocardiosis is restricted to *Nocardia* infections (Carter, 1973). However, earlier studies often referred to both *Actinomyces* and *Nocardia* infections as "actinomycosis" (Swerczak *et al.*, 1968); thus, review of the literature on this topic is quite difficult. Actinomycosis and nocardiosis are chronic, suppurative granulomatous diseases. *Nocardia* sp. produces subcutaneous lesions and soft tissue abscesses, but pulmonary involvement is the most frequent manifestation in humans. Sinus tract formation with dissemination is rare, but a tendency for hematogenous spread especially to the brain is occasionally reported (Murray *et al.*, 1960). *Actinomyces* sp. are frequently involved in subcutaneous, cervico-facial, thoracic, and abdominal infections (Conant *et al.*, 1971).

Sulfur granules frequently found in *Actinomyces* infections are not present in *Nocardia* infections. The granules in anaerobic *Actinomyces* infections are hard and gritty in texture, while those seen in *Nocardia* and *A. viscosus* infections are soft. The granules vary in size from a microscopic mass to a diameter of several millimeters. The cheese-like, yellow-gray granules of *A. bovis* and *A. israelii* contain colonies of filaments embedded in a polysaccharide-protein matrix with approximately

50% calcium phosphate. Sulfur granules are also found in maduromycosis, staphylococcal actinophytosis, and actinobacillosis. In hematoxylin and eosin stained histological sections, a mass of acidophilic, club-like projections radiate from the centers of the granules in a rosette pattern (Figures 1 and 2). This "club" formation is possibly a manifestation of an interaction of a cellular response of the host with the organism, not a product of the microorganism (Swerczak *et al.*, 1968). The center of the granule contains colony-like masses of rods and filaments which stain Gram positive. Sulfur granules are usually surrounded by neutrophils and then enveloped by lymphocytes, plasma cells, macrophages, and fibroblasts in fibroproliferative tissue (Swerczak *et al.*, 1968).

The granules found in nocardiosis are unlike the granules seen in bovine actinomycosis. These *Nocardia* granules lack club formations and appear to be a mass of organisms held together in a loose, unstructured, acidophilic matrix. The surrounding suppurative reaction is similar to that of actinomycosis (Kalnins, 1963). Swerczak *et al.* (1968) has described granules found in canine actinomycosis which he maintains are unlike *Nocardia* or bovine *Actinomyces* granules, but have a well defined border without clubbing. Anaerobic bacilli and various types of cocci were frequently found with *Actinomyces* sp. in the granules. Georg *et al.* (1968) also noted the absence of clubbed borders on granules found in mice which were inoculated intraperitoneally with *A. viscosus*.

Many *Actinomyces* sp. are saprophytes of the oral cavity of man and animals (Davis *et al.*, 1973). Only *A. humiferus* has been isolated from

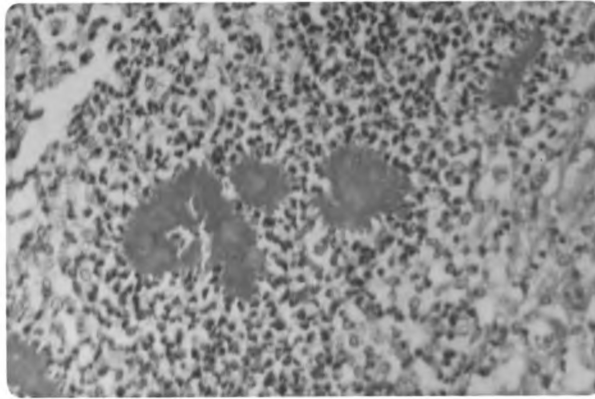


Figure 1. *A. bovis* sulfur granules with clubbed border. Hematoxylin and eosin stain; X 335.

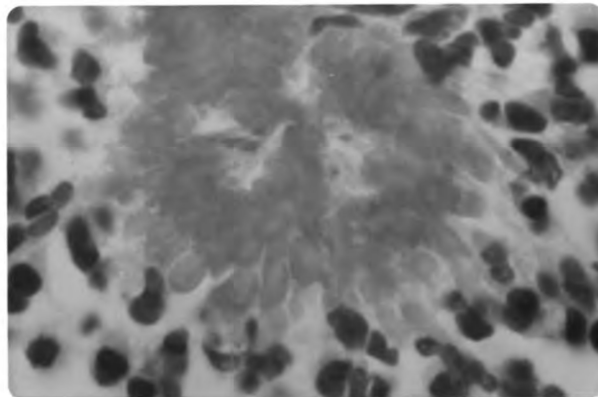


Figure 2. Higher magnification of *A. bovis* sulfur granules. Hematoxylin and eosin stain; X 1350.

the soil (Gledhill *et al.*, 1969). *Actinomyces eriksonii*, *A. israelii*, *A. bovis*, and *A. naeslundii* are commonly encountered in infections while *A. odontolyticus* and *A. viscosus* are considered nonpathogenic. Although *Nocardia* species are soil inhabitants, *Nocardia asteroides*, *N. brasiliensis*, *N. caviae*, *N. farcinica*, and *N. dassonvillei* have been isolated from purulogranulomatous nodular lesions (Thompson *et al.*, 1951). Although no cases of transmission of actinomycosis from man to man or from animal to man have been reported, caution should be observed by individuals handling infectious specimens.

Suspected modes of infection include trauma, passage through carious lesions in the gums, direct aspiration, or ingestion of the organisms in saliva. Lesions have been found in bones, thorax, abdomen, cecum, neck, skin, brain, tongue, soft tissues, and several organs (Conant *et al.*, 1971).

Cervico-facial actinomycosis can involve abscessation of soft tissue or bony hard tissue, including the maxilla or mandible. The soft granulomatous tissue contains necrotizing abscesses which coalesce to form draining sinuses or fistulous tracts (Georg *et al.*, 1968). Hard, dense tumorlike nodules simultaneously form in cutaneous tissue. The purulent exudate has a characteristic yellow-green color. Eventually fibrous scar tissue replaces regions of the granuloma. Osteitis from proliferation of the microorganism in the bone may cause a honeycombing effect due to rarefaction and proliferation of the bone. This may cause blockage of nasal passages and induce dyspnea. Dissemination of the organism to other tissues is effected by sinus tract formation. Sulfur granules are frequently found in cervico-facial actinomycosis (Smith *et al.*, 1972).

Aspiration is probably the usual mode of infection in thoracic actinomycosis. Symptoms may parallel those of pneumonia: irregular mild fever, cough, emaciation, anorexia, anemia, with subsequent production of bloody mucopurulent sputum, pleural pain, dyspnea, or abnormal respiratory sounds. Acute thoracic infections may cause pleural effusions with draining sinuses and fibrosis. Radiographs may reveal the spread of the infection from the base of the lungs (Hurst, 1950). Occasionally apical lung infections may resemble tuberculosis; however, no discrete circumscribed tubercule-like lesions will be apparent. Pleural adhesions and fluid accumulation may also be seen on x-rays. In pleural actinomycosis, mediastinal or other lymph nodes may become swollen and necrotic (Swerczak *et al.*, 1968). As fluid accumulates in the thoracic cavity, lung expansion is impeded. Pulmonary congestion, edema, and atelectasis gradually increase, and cardiac output may be reduced sufficiently to cause fluid accumulation in other body cavities which can debilitate the host and abet spread of the infection. Hyperplastic, fimbrionated villous proliferation of the thoracic wall causing pleural thickening is the result of a chronic tissue response to irritated pleural lining (Swerczak *et al.*, 1968).

Abdominal actinomycosis may result from ingestion of masses of contaminated saliva or from metastatic spread from cervico-facial or thoracic actinomycosis. Chronic abdominal dissemination to bones, liver, kidney, spinal column, lungs, and other regions has been reported. Weight loss, vomiting, fever, and abdominal pain are nonspecific symptoms which can also suggest appendicitis or abdominal carcinoma. Hepatomegaly, splenomegaly, and pyelonephritis may be apparent without

actual presence of infectious organisms in these organs. Any region of the gastrointestinal system may be infected with *Actinomyces* organisms. Suppurative burrowing abscesses and draining sinuses become embedded in fibrotic scar tissue, or are surrounded by yellowish lipid-filled macrophages. These regions should be examined for actinomycotic granules. Presence of granules excludes a diagnosis of syphilis, neoplasms, tularemia, amebiasis, disseminated tuberculosis, typhoid fever, granuloma inguinale, or certain deep-seated mycoses (Conant *et al.*, 1971).

Lesions due to different *Actinomyces* sp. cannot be differentiated. "Lumpy jaw" in cattle caused by *A. bovis* resembles a similar infection in humans due to *A. israelii*. Experimental infection of large animals with different species of actinomycetes has been rather unsuccessful. Even the most pathogenic actinomycetes, *A. bovis* and *A. israelii*, have induced infections in less than one-half of the inoculated test animals (Georg *et al.*, 1968).

Reported human infections with *A. naeslundii* include one abdominal abscess, one gallbladder empyema, three septicemias (one associated with leukemia and pyelonephritis), several sinus infections related to tooth extractions, one leg abscess, and one post-thyroidectomy wound abscess (Georg *et al.*, 1969a). Human infections with *A. viscosus* involved a branchial cyst abscess (Adeniyi-Jones *et al.*, 1973) and a case of pleural effusion complexed with tuberculosis. Georg *et al.* (1972) isolated *A. viscosus* from one case of enzootic pneumonia in pigs, a lymph node abscess in a goat, and two cases of abdominal infections in dogs. Canine strain 1231 was isolated from an abdominal mass attached

to the pancreas, stomach, spleen, intestines, and kidneys of a six-month-old terrier. The dog was presented with signs of emaciation, vomiting and general malaise. Granules found in a biopsy had irregular margins but no clubbed edges. Granules yielded *Eubacterium lentum*, *Enterobacter cloacae* and *A. viscosus* in culture. The latter two organisms are considered normal intestinal flora.

A six-month-old mixed breed dog was presented with signs suggestive of abdominal effusion: lassitude, anemia, emaciation, abdominal distention, pale oral mucosa, hepatomegaly, and a mild leukocytosis with a shift to the left. No sulfur granules were seen; however, numerous abscesses were observed on the liver. The latter were surrounded by yellow zones of lipid accumulation, fibrotic tissue, and hemorrhagic material. On histologic examination the center of the abscesses contained mostly dying hepatocytes and colonies of *A. viscosus* filaments.

Nocardiosis is a disease found in cattle, man, dogs, cats, marsupials, horses and goats, and tropical fish (Bruner *et al.*, 1973). Subcutaneous, abdominal and thoracic forms have been reported in varying frequencies from different animal species. Subcutaneous abscesses, empyema, pyopneumothorax and infections of the stomach, brain, kidneys, bones and heart have been reported in dogs (Bruner *et al.*, 1973). Definitive diagnosis requires cultural identification of the organisms since lesions closely resemble those seen in actinomycosis. There is frequently extensive vascularization of lesions particularly in villous proliferation of serosal surfaces. Non-clubbed flake-like granules containing filaments may aid in a tentative diagnosis if the organism is partially acid-fast.

Confusion of *A. viscosus* with Other Organisms

Reported infections with *A. naeslundii*, *A. viscosus* and *N. asteroides* have similar pathological and morphological characteristics. *Nocardia* sp. appeared as the cause of most Gram-positive filamentous infections in dogs seen at the Michigan State University Veterinary Clinic during the past eight years (Table 2). These statistics, however, are somewhat weighted since all Gram-positive, aerobic, filamentous organisms were thought to be *N. asteroides* until 1973, when knowledge of more precise techniques for identification of Actinomycetales were applied. Failure to differentiate *A. viscosus* from *N. asteroides* probably accounts for the low incidence of reported cases of canine actinomycosis. Not listing an acid-fast stain, improper decolorization of the stain, and knowledge that some *Nocardia* species are non-acid-fast may result in some microbiologists reporting all Gram-positive, aerobic, catalase-positive filamentous organisms as *N. asteroides* (Georg *et al.*, 1969b).

The absence of clubs in granules of *A. viscosus* has not been reported in most literature on actinomycosis; however, many references are found which cite the presence of non-clubbed flaky granules in nocardiosis (Langham *et al.*, 1959; Murray *et al.*, 1960), thus causing further confusion in identification of these two potential pathogens (Swerczak *et al.*, 1968). Diphtheroidal bacteria may be a contaminant or, on occasion, a pathogen (Johnson *et al.*, 1970). A diphtheroidal non-filamentous form of *A. viscosus* also exists which physiologically and morphologically resembles these bacteria (Adeniyi-Jones *et al.*, 1973).

Table 2. Canine and feline cases from which filamentous microorganisms were isolated in the Clinical Microbiology Laboratory, Veterinary Clinic, Michigan State University, from 1965 to 1974

Date	Breed	Clinic Case No.	Clinical Diagnosis	Microbiology Case No.	Cytology Smear	Histopathology	Microbiology Report	Microbiology Data	Granules
9/64	German Shepherd	102932	Submandibular nocardiosis abscess	C-849-64	0	0	N*	<i>Mina polymorpha</i> cultured N	
1/65	Domestic Feline	100677	Abd. effusion nocardiosis	F-2-65	N	N	N	Gram stain (GS) of BA culture	Sm. soft gran.
3/65	Brittany Spaniel	104449	Hip abscess nocardiosis	C-179-65	N	-	N	GS of BA culture	
4/65	Collie	104600	Generalized nocardiosis	C-211-65 C-226-65	0	-	N	<i>St. aureus</i> GS of BA culture	
5/65	Labrador Retriever	104973	Thoracic nocardiosis distemper	not reported	0	-	N		
12/65	Coonhound	107384	Thoracic nocardiosis	C-977-65	N	0	-	<i>β Streptococcus</i> , <i>Proteus</i> , <i>P. multocida</i> , <i>E. coli</i> , <i>St. aureus</i>	
1/66	Walker Hound	107601	Thoracic actinomycosis	C-32-66	N	-	<i>Actinomyces</i>	No growth on aerobic BA, GS of anaerobic BA culture	

Table 2 (continued)

Date	Breed	Clinic Case No.	Clinical Diagnosis	Micro- biology Case No.	Cytol- ogy Smear	Histo- path- ology	Micro- biol- ogy Report	Microbiology Data	Granules
1/66	German Shepherd	107824	Nocardiosis or actinomycosis empyema	C-65-66 C-84-66	0	N	<i>Actino- myces</i>	Poor growth on aerobic BA, GS of anaerobic BA culture	
1/66	German Shepherd	100013	Subcut., lung and sp. cord nocar- diosis	C-446-66 C-543-66 C-548-66 C-581-66	-	-	-	γ <i>Streptococcus</i> , β <i>Streptococcus</i> , <i>Micrococcus</i> , <i>P.</i> <i>multocida</i> , <i>E. coli</i> , <i>St. aureus</i>	
7/66	Boxer	109456	Submandibular nocardiosis abscess	C-419-66	0	0	N	<i>Bacteroides</i> , anaerobic <i>Strep.</i> GS of anaerobic BA culture	
10/66	Poodle	107808	Cervical abscess nocardiosis		0	0	0		
3/67	Siberian Husky	112297	Peritonitis nocardiosis	C-225-67 C-236-67	0	N	N	<i>E. coli</i> , <i>Sal- monella</i> ; N = indol + sucrose + dextrose + (gas)	Gran.
5/67	Labrador Retriever	113028	Possible nocardio- sis abscess	C-407-67	0	-	-	<i>St. aureus</i> , <i>Clostridium</i>	

Table 2 (continued)

Date	Breed	Clinic Case No.	Clinical Diagnosis	Micro-biology Case No.	Cytology Smear	Histopathology	Microbiology Report	Microbiology Data	Granules
10/67	Scottish Terrier	114965	Lumbar fistula nocardiosis	C-839-67 C-849-67	-	-	N	Acid-fast culture	
11/67	Dachshund	115329	Generalized nocardiosis	C-420-67 C-922-67	-	-	N	N = GS of culture BA; diphtheroid also found with these reactions: urea -, catalase +, dextrose +, NO ₃ -, motility -	
1/68	Tick Hound	120329	Pyothorax nocardiosis	C-14-69	N	N	N	<i>E. coli</i> , <i>Bordetella</i> , N grew on BA, did not grow on SAB	
5/68	St. Bernard	117800	Cervical cyst nocardiosis	C-459-68	N	N	N	GS only of exudate could not grow	
8/68	Walker Hound	119274	Thoracic granuloma nocardiosis	C-679-68	N	N	-	<i>St. aureus</i>	
2/69	Brittany Spaniel	120981	Empyema nocardiosis	C-159-69 C-188-69	N	0	-	<i>P. multocida</i> , <i>Pseudomonas</i> GS	
3/69	Dalmatian	121041	Flank fistula, probable nocardiosis		0	<i>Actinomyces</i>	0	of tissue	

Table 2 (continued)

Date	Breed	Clinic Case No.	Clinical Diagnosis	Micro-biology Case No.	Cytology Smear	Histopathology	Microbiology Report	Microbiology Data	Granules
3/69	German Shepherd	121285	Pyothorax nocardiosis	C-228-69	N	0	N	GS of BA culture did not grow on SAB	
4/69	German Shepherd	118111	Pyothorax nocardiosis	C-312-69 C-605-71	N	0	N	<i>P. multocida</i> , <i>Bacteroides</i> N = GS of tissue fluid	
7/69	Walker Hound	122954	Salivary cyst granuloma nocardiosis		0	-	0		
9/69	English Pointer	123600	Pyothorax nocardiosis	C-878-69 C-885-69	-	N	-	<i>St. aureus</i> could not culture	Yellow gran.
12/69	Beagle	124752	Submaxillary abscess nocardiosis	C-1117-69	-	N	N	Culture GS non-acid-fast	
1/70	Walker Hound	121810	Osteomyelitis nocardiosis	C-405-69	N	-	N	GS of discharge could not culture	
2/70	English Setter	125329	Cervical granuloma nocardiosis	C-137-70	N	0	N	GS of culture BA did not grow on SAB	
4/70	Malamute	126286	Cervical abscess nocardiosis	C-501-70	-	-	N	<i>St. aureus</i> GS of discharge could not culture	

Table 2 (continued)

Date	Breed	Clinic Case No.	Clinical Diagnosis	Micro- biology Case No.	Cytol- ogy Smear	Histo- path- ology	Micro- biol- ogy Report	Microbiology Data	Granules
5/70	English Pointer	126659	Empyema nocardiosis	C-576-70 C-580-70	0	N	N	β <i>Streptococcus</i> GS of BA culture no growth on SAB	Yellow gran.
6/70	St. Bernard	127098	Mandibular abscess nocardiosis	C-706-70	N	0	-	<i>Corynebacterium</i> , <i>St. aureus</i> , γ <i>Strep.</i> , <i>St.</i> <i>epidermidis</i> , <i>P.</i> <i>multocida</i>	
7/70	English Setter	129107	Pyothorax nocardiosis	C-786-70	N	-	N	GS of BA culture no growth on SAB non-acid-fast	
10/70	St. Bernard	129221	Cervical abscess nocardiosis	C-1067-70	N	0	-	could not grow	
11/70	Malamute	129221	Salivary cyst nocardiosis	C-1087-70	N	+	-	could not grow in culture	
12/70	Collie	129861	Granulomatous meningoencephalitis nocardiosis		0	Possible N	0		
1/71	Labrador Retriever	129059	Submaxillary abscess nocardiosis	C-1199-70	-	-	N	GS of BA culture	
2/71	St. Bernard	120971	Cervical abscess nocardiosis	C-49-71	N	0	N	<i>Bacillus</i> , <i>Micro-</i> <i>coccus</i> GS of exudate only	Gran.

Table 2 (continued)

Date	Breed	Clinic Case No.	Clinical Diagnosis	Micro- biology Case No.	Cytol- ogy Smear	Histo- path- ology	Micro- biol- ogy Report	Microbiology Data	Granules
4/71	Leopard Kerr	130461	Cervical granuloma	C-191-71	N	0	-	no growth	
9/71	Red Bone	133881	Temporal abscess nocardiosis	C-605-71	N	0	-	no growth	
12/71	Cocker Spaniel	134856	Granuloma nocardiosis	C-797-71	N	-	-		Gran.
2/72	Great Dane	134321	Cervical abscess nocardiosis	C-806-71	N	0	-	no growth	
4/72	Great Dane	131712	Mandibular granu- loma nocardiosis	C-235-72 C-251-72	0	N	-	<i>Micrococcus</i> , γ <i>Streptococcus</i>	Clubbed gran.
6/72	Great Dane	129820	Cervical granu- loma nocardiosis	C-470-72	N	0	N	Non-acid-fast no growth on SAB, urea -, gel -	
11/72	St. Bernard	133352	Mandibular abscess nocardiosis	C-797-72 C-818-72 C-850-72	-	-	-	<i>St. epidermidis</i> , <i>Proteus</i> , <i>Micro-</i> <i>coccus</i> , β <i>Strep.</i> , <i>St. aureus</i> , γ <i>Strep.</i> , <i>Pseudo-</i> <i>monas aeruginosa</i>	

Table 2 (continued)

Date	Breed	Clinic Case No.	Clinical Diagnosis	Micro- biology Case No.	Cytol- ogy Smear	Histo- path- ology	Micro- biol- ogy Report	Microbiology Data	Granules
11/72	St. Bernard	139713	Cervical granuloma nocardiosis	C-908-72	N	N	N	<i>Micrococcus</i> GS of BA culture	
1/73	Domestic Feline	127784	Pleuritis nocardiosis	FD-10-73	0	N	N		
1/73	Labrador Retriever	138631	Temporal abscess actinomycosis	C-99-73	N	-	<i>A. vis- cosus</i>	<i>Pasteurella, Moraxella, Alcal- igenes fecalis</i> numerous tests performed	
2/73	Collie	140591	Periorbital abscess nocardiosis	C-132-73	N	0	N	Non-acid-fast exudate could not grow in culture	
3/73	English Setter	141564	Empyema actinomycosis	C-295-73	N	0	<i>A. vis- cosus</i>	<i>Cl. perfringens</i> numerous tests performed	
5/73	English Pointer	131568	Flank abscess nocardiosis or actinomycosis	C-265-71 C-492-73	<i>Actino- myces</i> or <i>Nocardia</i>	-	N <i>A. vis- cosus</i>	265 - no growth on SAB, GS of BA cul- ture - <i>Nocardia</i> ; 492 - extensive tests - <i>A. viscosus</i>	

Table 2 (continued)

Date	Breed	Clinic Case No.	Clinical Diagnosis	Micro- biology Case No.	Cytol- ogy Smear	Histo- path- ology	Micro- biol- ogy Report	Microbiology Data	Granules
8/73	Mixed	144302	Hip fistula nocardiosis	C-769-73	N	-	-	<i>Cl. perfringens</i> , <i>Micrococcus</i> , <i>Mima</i> <i>polymorpha</i>	
11/73	Irish Setter	145419	Right flank abscess	C-1004-73	N	-	-	no growth	
11/73	Walker Hound	145833	Empyema actinomycosis	C-1078-73 C-1097-73	N	N	<i>A. vis- cosus</i>	<i>K. pneumoniae</i> <i>A. viscosus</i> = numerous tests performed	
1/74	Norwegian Elkhound	147495	Cervical actinomycosis	C-194-74	-	<i>Actino- myces</i>	<i>A. vis- cosus</i>	<i>A. viscosus</i> = numerous tests performed	

* N = *Nocardia*; - = negative; 0 = not done; BA = blood agar, SAB = Sabouraud's agar; gran. = granules.

Treatment of Canine Actinomycosis and Nocardiosis

Because there have been so few cases of canine actinomycosis diagnosed, there is very little information on the efficacy of treatment. *Actinomyces viscosus* is sensitive to most antibiotics. Penicillin is the drug of choice. Chloramphenicol, oxytetracycline, erythromycin, clindamycin, ampicillin, and chlortetracycline are also very effective (Lerner, 1974). Streptomycin, rifampin, and novobiocin are not as potent against these infections as many broad-spectrum antibiotics (Adeniyi-Jones *et al.*, 1973; Murray *et al.*, 1960; Davis *et al.*, 1973). The latter drugs are usually given intravenously or orally for two or more weeks until no signs of active infection persist. Surgical excision of infected tissues and drainage of wounds cannot adequately eliminate infection from fibrotic scar tissue unless antibiotics are also used. Topical application or flushes with iodine solution are recommended in treatment of subcutaneous abscesses. Antibiotic therapy has not been too successful in progressive cases of canine actinomycosis when there is extensive damage to organs and development of fistulous tracts. Cure depends upon early diagnosis and treatment of the infection.

In vivo Nocardia infections are resistant to penicillin and other antibiotics. Treatment usually includes sulfonamide drugs, but broad-spectrum antibiotics, streptomycin, novobiocin, or erythromycin are often prescribed along with the sulfonamides (Murray *et al.*, 1968; Kalnins, 1963. Treatment of nocardiosis may have to be carried out for several weeks. Advanced infections respond poorly to antibiotic

therapy, so veterinary clinicians frequently recommend euthanasia.
Thus proper diagnosis of the etiologic agent is very important.

CLINICAL HISTORIES OF CANINE ACTINOMYCOSIS SEEN AT THE MICHIGAN
STATE UNIVERSITY VETERINARY CLINIC JUNE 1972 TO NOVEMBER 1973

Clinic Case 129820 (CF-470-72)

The referring veterinarian had been unsuccessful in three weeks of treatment of an abscess on a two and one-half-year-old male Great Dane. Eighteen months earlier the dog had been diagnosed as having bilateral osteochondritis dissecans of the humeral heads. No treatment for osteochondritis had been initiated. Inflamed tonsils and a cervical swelling were noted during the preliminary examination at the Veterinary Clinic on June 5, 1972. Tentative diagnosis suggested a salivary cyst. The enlarged mass was below the right cervical region, posterior to the mandible. The pathologist examined large, soft, mucoid granules present in the infected tissue and noted the presence of Gram-positive, filamentous, "*Nocardia*-like" microorganisms. The microbiologist cultured a "probable *Nocardia* species" which was non-acid-fast, urease negative, gelatinase negative, and did not grow on Sabouraud's agar. A diagnosis of nocardiosis was made.

The mass was excised, drained and flushed daily with Betadine solution,¹ and Bactro-Vet² was administered orally. Radiographs of the chest did not indicate thoracic infection. The University Clinic and

¹Providone-Iodine, Purdue-Frederick.

²Sulfadimethoxine, Pitman-Moore.

the referring veterinarian have had no further admissions with this animal.

Suggested route of infection through the mouth, presence of mucoid granules, and atypical cultural reactions left some doubt in the final diagnosis of nocardiosis. The strain was sent to Dr. L. K. Georg at CDC in Georgia for positive identification. The strain was dead when it arrived at CDC, but fluorescent antibody studies and catalase reaction were examined. Georg said this strain was catalase-negative and did not react with *A. viscosus* fluorescent antibody. At that time, information on identification of *A. viscosus* was brought to the attention of the microbiologist and pathologist at the Veterinary Clinic.

Clinic Case 138631 (CF-99-72)

This seven and one-half-year-old female Labrador Retriever had a history of false pregnancy, ovariohysterectomy, epilepsy, and a single episode of dyspnea due to inhalation of phlegm during one of the seizures. Slightly elevated blood sugar, 116 mg/100 ml, and a blood urea nitrogen of 22-24 mg% were also noted. Medication included Mebroin¹ and Myelepsin² prescribed by the family veterinarian for seizures, later substituted with Dilantin³ and Primidone⁴ prescribed by the MSU Veterinary Clinic. The family veterinarian had administered

¹Diphenylhydantoin, Winthrop.

²Primidone, Fort Dodge.

³Diphenylhydantoin, Parke, Davis & Co.

⁴Primidone, Ayerst.

broad-spectrum antibiotics as treatment for a temporal abscess which appeared on the animal during hunting season. The abscess seemed to have started on the nose and moved upwards towards the left eye causing a swelling on the left side of the head. Initially the swelling was firm but, after several weeks of intermittent treatment, it softened. Purulent material was aspirated from the masseter muscle region.

The abscess was present for two months when first examined at the MSU Veterinary Clinic on January 1, 1973. This involved the left temporal and periorbital areas, causing the left eye to be nearly swollen closed. Part of the mass was fluctuant, part firm, and the entire mass was very painful and quite warm to touch. Poor pupillary response in the right eye, enlarged cervical lymph nodes, and dandruff were also noted during the initial examination. Marked eosinophilia, $1738/\text{mm}^3$, and a slight increase in blood urea nitrogen, 22 mg%, were among the laboratory findings. Pus from the abscess contained numerous macrophages and polymorphonuclear leukocytes, in which abundant Gram-negative bacilli, Gram-positive bacilli, and Gram-positive pleomorphic beaded filamentous microorganisms were seen. An acid-fast stain was negative; however, the pathologist remarked that the filaments were suggestive of *Nocardia* species. Heavy growth of *Actinomyces viscosus* and a lighter growth of *Alcaligenes fecalis*, *Mima polymorpha* var. *oxidans*, and a *Pasteurella* species were cultured from the exudate.

Chloramphenicol succinate administered both subcutaneously and orally caused a marked reduction in the size of the temporal mass within approximately two weeks, despite the presence of a chloramphenicol

resistant strain of *Mima polymorpha*. The abscess was lanced and drained, and the dog was discharged from the clinic on January 26. Chloramphenicol and Primidone therapy were continued after discharge.

Follow-up examination one week later revealed the mass had shrunk to about one inch. Surgical removal of fibrotic tissue and a fistulous tract which extended to the skull bones and curved towards the maxilla was undertaken. Chloramphenicol was continued for another week. Except for drainage of surgical seromas, no further complications or recurrence of infection have been reported.

Clinic Case 141564 (CF-295-73)

A three-year-old male English Setter with a history of hip problems resulting from being struck by a car was admitted to the Clinic on March 6, 1973. The dog had been emaciated and dyspneic for the preceding two months. The symptoms seemed to appear at the end of an active hunting season. Hookworms, mild fever (104 F.) and problems with urination had been reported by the referring veterinarian, who had x-rayed the chest region and had been treating the dog with tetracycline. Radiographs revealed an obscured mediastinal area which suggested fibrotic attachments forming adhesions. Mild anemia with a hemoglobin value of 10 mg% and a packed cell volume of 30% was evident.

Preliminary examination at the Clinic revealed abnormal lung sounds, deformed hips, pale mucous membranes, and anemia. A normal white blood cell differential count with a slight terminal increase in monocytes was reported. Thoracic fluid was aspirated and examined microscopically. This contained a moderate number of degenerative

polymorphonuclear leukocytes and macrophages. Microorganisms seen in the fluid included coccobacilli, cocci, and Gram-positive branching filaments "morphologically identical to *Nocardia asteroides*." *Actinomyces viscosus* and *Clostridium perfringens* were grown in culture from the exudate.

A series of radiographs were taken. The first showed compressed lungs floating on top of serosanguineous exudate. When the dog was in a prone position the lungs remained in the dorsal region, apparently attached there by adhesions. There was a moderate thickening of the pleura probably due to villous proliferation and fibrosis. Gunshot-like objects were visible in the dorsal area near the spine. The lungs remained clear. After large amounts of fluid were aspirated from the thorax, radiographs again were taken which demonstrated slight clearing of the thoracic cavity; however, some fluid was still evident. One week later, radiographs showed that the condition had stabilized, but pleural thickening and adhesions were still prominent. The expansion ability of the lungs was restricted to about 60% because of these adhesions. A diagnosis of pyothorax due to *A. viscosus* was made.

Treatment included oral tetracycline during the first and fourth weeks of therapy. Daily injection of penicillin-streptomycin intrathoracically and Combiotic¹ intramuscularly was maintained for about three and one-half weeks. Response to antibiotic therapy was

¹Penicillin, dihydrostreptomycin, Pfizer.

excellent, and the dog was discharged from the Clinic on April 11, 1973. The local veterinarian prescribed sulfadimethoxine for three weeks. One year later the dog was reported to be in good condition. He participated in a successful active hunting season during the fall of 1973. Fibrotic tissue was most likely reduced and lung capacity increased.¹

Clinic Case 131568 (CF-492-73)

A five-year-old male English Pointer was presented with a small flank abscess in January 1971. The local veterinarian lanced and drained the abscess on January 17 and February 5, and penicillin was administered orally and by injection. The dog was presented with multiple abscesses in the flank area February 25. The abscesses were drained, penicillin was administered by injection, and tetracycline was given orally. The animal was admitted to the Veterinary Clinic on March 10, with multiple flank abscesses and a circumscribed swelling on the left thorax. The general condition of the animal was fair. Radiographs of the left flank region showed an increase in density of the soft tissue, suggestive of swelling; however, no evidence of fistulous tracts was apparent. Injection of sodium iodide through a polyethylene catheter into the abscesses revealed two fistulous tracts in the next series of radiographs, one anterior to the left femur and one anterior to this, extending to the thirteenth rib. These tracts were confined to subcutaneous and muscular layers of the abdominal wall, apparently ending retroperitoneally in the region of the kidneys.

¹Personal communication, Dr. R. G. Schirmer.

Chest radiographs showed a normal thoracic cavity with the exception of what appeared to be three small lead pellets embedded in subcutaneous tissue.

Laboratory reports included isolation of *Staphylococcus aureus* and *Nocardia* sp. (the latter did not grow on Sabouraud's agar) from flocculent material in the serosanguineous aspirate and the presence of large numbers of hookworm and whipworm ova in the feces. Normal levels of hemoglobin, total protein, blood urea nitrogen, and packed cell volume were noted. Blood cell differential counts showed a slight increase in nonsegmented neutrophils, 420-704/mm³. The body temperature was 102 F. The abscess was excised and drained. Histological examination of excised tissue showed increased vascularization, suppuration, scar tissue and fibrotic connective tissue in which foci of macrophages, plasma cells and lymphocytes were present. No organisms were seen in the smears, but the pathology report mentioned the presence of *Nocardia* sp. (beaded, Gram-positive filaments) in white flakes found in the exudate.

Following surgery, the wounds were flushed daily for several days with Betadine solution. Oral administration of tetracycline, chloramphenicol, and ampicillin was prescribed sequentially for two weeks, and the dog was then discharged with a 10-day prescription of Bactro-Vet.

The animal was readmitted on April 23, 1971, for surgical debridement of a newly formed abscess in the same area. The fistulous tract was again opened and granulomatous material was excised. Pathological examination again revealed filamentous beaded organisms "suggestive of *Nocardia*." The blood differential appeared essentially normal. The

animal was treated with oral tetracycline for one month, and the wound was flushed daily with Betadine solution.

The last admission of this dog to the Veterinary Clinic was on May 8, 1973. Reported fluctuation under the left flank and right rib cage associated with severe pain suggested regression. Occasional mucoid stool production and a slight abnormal gurgling or crepitation in the left dorsal region of the lungs was reported. Radiographs revealed a subcutaneous swelling over the rib cage of the right side. No internal thoracic lesions were noted and the lungs remained clear. The cause of a fibrotic change at the eleventh, twelfth and thirteenth thoracic vertebrae could not be explained. Urinary tract function remained normal. Marked eosinophilia, $1848/\text{mm}^3$, and basophilia, $420/\text{mm}^3$, were apparent five days after surgical debridement. Wright's stains of infected tissue showed large numbers of macrophages, neutrophils, and occasional eosinophils and lymphocytes.

Cultural studies provided positive identification of *Actinomyces viscosus*. This led the clinician to the conclusion that earlier isolates were also *A. viscosus* since earlier cultural techniques were inadequate for definite identification. Methods for differentiation of *Actinomyces* and *Nocardia* were not made known to the laboratory until 1973. The dog was again treated with oral tetracycline and Betadine flushes for 10 days. No further remissions have been reported.

Clinic Case 145833 (CF-1078-73, CF-1097-73)

A six-year-old male Walker Hound with signs of labored breathing and thoracic empyema was admitted to the Clinic on November 3, 1973. The animal had been recently treated for diarrhea with administration

of chloramphenicol and Kaopectate.¹ Radiographs revealed an increase in opacity of the thorax.

In the same kennel a hound had died one month earlier. This hound suffered with an acute onset of dyspnea, malaise, and fluid in the thorax. Necropsy findings included the presence of red-tinged fluid in the pleural cavity. The lungs were consolidated, only 50% functional, and were covered with white patches suggestive of a possible fungal infection. No cultural studies were performed.

The preliminary examination of this second Walker Hound suggested a tentative diagnosis of pneumonia. The animal was emaciated and dyspneic, and moist rales were heard in the dorsal area of the thorax. No sounds were heard in the ventral thorax, suggesting fluid accumulation. Blood cell differential indicated an inflammatory reaction: leukocytes 14,100, monocytes $423/\text{mm}^3$, eosinophils $423/\text{mm}^3$, lymphocytes $2115/\text{mm}^3$, segmented neutrophils $1551/\text{mm}^3$, nonsegmented neutrophils $9588/\text{mm}^3$, 2 nucleated erythrocytes/100 leukocytes.

Fluid was aspirated from the thorax and examined microscopically. It consisted of large numbers of neutrophils and fewer mononuclear cells which had phagocytized Gram-positive beaded and branching filamentous organisms. The pathologist suggested the filaments resembled the *Nocardia*-like microorganism similar to ones seen in other cases of canine pleural effusions. Radiographs revealed fluid in the abdominal and thoracic cavities. Pleural thickening was seen and changes in interstitial areas in the lung were questioned. The possible

¹Kaolin, Pectin, Upjohn.

interstitial changes could have been indicative of consolidated, patchy granulation tissue.¹

The animal became more dyspneic and died five days later. Necropsy reports suggested a diagnosis of a *Nocardia* infection. Thoracic and abdominal cavities were distended and filled with fluid. Extensive villous proliferation in the pleura and pericardial sac, suggestive of a chronic infection, showed progressive replacement with granulation tissue. Only a small dorsal portion of the lung had not collapsed. The foul-smelling, red-tinged exudate restrained the lungs thus impairing expansion and contraction. This induced gradual atelectasis. Abundant gray-white, soft, sulfur-like granules were found in the lung (Figure 3). These ranged from one millimeter to five millimeters in diameter. There was a conspicuous lack of clubbing

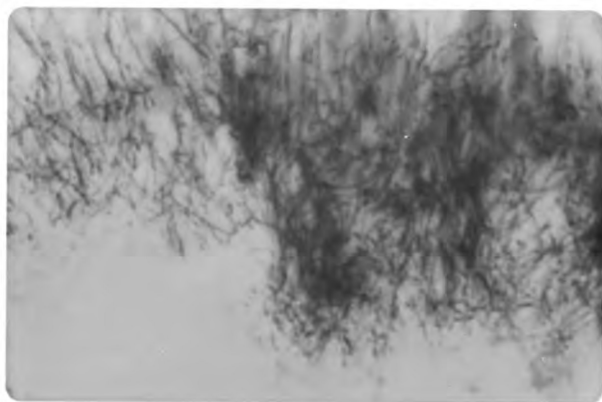


Figure 3. CF-1097-73. Histopathology of *A. viscosus* granules from lung tissue. Gram's stain; X 1350.

around the border of these granules, and filamentous microorganisms were dispersed throughout the granules. No granules were found in the abdominal tissue or in the white-brown abdominal fluid. Hepatomegaly was observed, but the cause of enlargement was not investigated.

¹Personal communication, Dr. R. G. Schirmer.

Microbiological examination of thoracic fluid, specimen 1078, and lung tissue, specimen 1097, revealed *A. viscosus* as the filamentous organism. Gram-stained granules indicated the presence of Gram-negative bacilli and Gram-positive cocci which did not grow in culture. A diagnosis of pyothorax due to *A. viscosus* was made.

MATERIALS AND METHODS

Organisms

Four cultures of *Actinomyces viscosus* Strains CF-295-73, CF-470-72, CF-492-73, CF-99-72 were obtained from Mr. H. McAllister, who cultured purulent material on trypticase soy agar (BBL) with 5% bovine blood. Strain CF-1097-73 was cultured from granules obtained from lung tissue of a dog with pyothorax and strain CF-1078-73 was cultured from thoracic fluid aspirated from the same animal.

Culture Media

Media were prepared anaerobically on the Virginia Polytechnic Institute (VPI) Anaerobic Culture Media Apparatus (Bellco, Inc.) using the procedure described in the VPI manual (Holdeman *et al.*, 1972). The Hungate-roll tube (Hungate, 1950) was not used since colonial morphology is difficult to observe in these tubes. Instead, flat-bottomed 100 ml prescription bottles were filled with 15 ml of media, under an atmosphere of 90% nitrogen and 10% carbon dioxide, and a rubber stopper sealed the opening of the bottle. This anaerobic apparatus provided a constant flow of oxygen-free gas during preparation of media and transfer of cultures. Traces of oxygen were removed by passage of gas through a cold catalyst (Dexco). The Eh value of media prepared by this method is about 150 mv. Liquid media were prepared

anaerobically on the same apparatus by the techniques described by Holdeman *et al.* (1972), dispensed in 5 ml portions into 18 mm tubes and capped with rubber stoppers. Aerobic media were of the same composition as anaerobic media but lacked the reducing agent, cysteine hydrochloride, and the Eh indicator, resazurin. Studies of aerobic growth in an increased carbon dioxide atmosphere were performed by placing aerobic cultures in a torbal jar, then evacuating and flushing with carbon dioxide. All cultures were examined after incubation at 37 C.

Isolation Methods

Primary isolation from clinical material was made by crushing granules washed with saline solution and streaking the material onto anaerobic and aerobic supplemented BHI-blood agar,¹ while the exudate was directly streaked onto similar media. Gram stains of the crushed granules and exudate were prepared and then examined microscopically. Specimens were then frozen at -40 C to preserve for future use.

Preservation of Stock Cultures

Seven-day stock cultures were preserved in 0.5 ml defibrinated blood, fast frozen with dry ice and stored at -40 C. Cultures were also suspended in mist dessicans² after 10 days' inoculation on supplemented BHI-blood agar and then lyophilized.

¹Five percent bovine ox blood in Difco BHI agar supplemented with vitamin K-hemin solution and yeast extract (Holdeman *et al.*, 1972).

²One part 6% glucose solution, 3 parts horse serum, 1 part BHI broth.

Morphologic Studies

Strains of *A. viscosus* were inoculated on supplemented BHI agar, supplemented BHI-blood agar, then incubated at 37 C under aerobic, aerobic with CO₂, and anaerobic with CO₂ conditions. Colonial morphology was examined at 24 hours, 48 hours, 7 days, and 14 days with a dissecting microscope at 40X. Microscopic examination of colonies from supplemented BHI-blood agar incubated anaerobically with CO₂ and aerobically with CO₂ was performed after growth at 48 hours and 7 days. Supplemented BHI broth¹ cultures were examined at 24 hours', 48 hours' and 7 days' incubation under aerobic with CO₂ and anaerobic with CO₂ atmospheric conditions. Gram stains of 48-hour and 7-day broth cultures were examined microscopically. The amount of growth was estimated visually as trace to 4+.

A Kinyoun modified acid-fast stain (Georg *et al.*, 1961) was performed on 4-day cultures grown aerobically with CO₂ on supplemented BHI-blood agar. Control stains of *Nocardia brasiliensis* and *Streptomyces madurae* were stained for comparison with the *Actinomyces* specimens.

Biochemical Tests

Biochemical tests were done by the procedures recommended by Holdeman *et al.* (1972) using a basal liquid medium of peptone yeast extract with 5% or 10% carbohydrate concentration for carbohydrate fermentation tests. Carbohydrate media were dispensed in 5 ml amounts

¹Difco BHI broth supplemented with vitamin K-heme solution (Holdeman *et al.*, 1972).

into test tubes and autoclaved at 110 C for 12 minutes. Carbohydrates in a concentration of 1.0% included: cellobiose, dulcitol, fructose, galactose, glucose, inositol, inulin, lactose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol, starch, sucrose, and xylose. Those diluted to a final concentration of 0.5% were: adonitol, arabinose, melibiose, ribose, and trehalose. Reagents and media for indol production, catalase, nitrate reduction, methyl red reaction, starch hydrolysis, gelatin hydrolysis, urease production, acetylmethylcarbinol (Voges-Proskauer) production, and aesculin hydrolysis tests were prepared according to the techniques described in the *Anaerobic Laboratory Manual* (Holdeman *et al.*, 1972). Bacto litmus milk was prepared according to directions provided by the manufacturer. Inoculum for all tests was made from 2-day-old cultures grown aerobically with CO₂ on supplemented BHI-blood agar. A heavy suspension of *A. viscosus* was prepared by mixing the culture in physiological saline, and four drops were added from a Pasteur pipette to each tube of medium. The litmus milk tube received seven drops of inoculum to assure a strong visible reaction. All tubes were incubated in a candle jar for 10 days at 37 C.

Carbohydrate fermentation was determined by measuring the final pH of the media on a pH meter (Model 265 Instrumentation Laboratory, Inc.). A pH of 6.2 or lower was considered positive, and 6.3 or above was a negative reaction. Ribose, xylose, and arabinose had to have a pH reading lower than 5.7 before a reaction was positive, since CO₂ tends to lower the pH of this medium (Holdeman *et al.*, 1972). Interpretation of results of other biochemical tests was by standard

procedures described in many manuals (Holdeman *et al.*, 1972). All tests were repeated once. Isolates which did not give results consistent with those reported in literature (Table 3) were again repeated using freshly made test media.

Carbon Dioxide Requirements

The basal peptone yeast extract was prepared under pure nitrogen gas which had been passed through sodium hydroxide pellets to remove trace amounts of carbon dioxide. Procedures for inoculation of media and preparation techniques were similar to those described in preparation of anaerobic media with CO₂. Growth was examined after seven days and estimated visually as trace to 4+. Comparison of aerobic growth, with aerobic plus CO₂ growth, was made on seven-day broth cultures, supplemented BHI agar and supplemented BHI agar with 5% bovine blood described in the section on morphological studies (page 52). Estimated value of carbon dioxide in the atmosphere is .03% (Spector *et al.*, 1956). However, presence of technicians and CO₂ containing apparatus in the laboratory may have increased the concentration to 0.5% CO₂. Carbon dioxide added to the torbal jars was not quantitated, but more than 10% was estimated to be delivered.

RESULTS

Morphological Characteristics

All strains of *Actinomyces viscosus* were Gram-positive, diphtheroidal rods or filaments, and were non-acid-fast. A slight preference for supplemented BHI agar with blood over supplemented BHI-agar was seen. However, BHI agar supported much better growth of *A. viscosus* than trypticase blood agar. No strains grew on Sabouraud's agar. Two morphological types were seen: a smooth entire convex glistening mucoid form (Strains CF-295-73, CF-492-73, CF-99-72, CF-1078-73) and a dry granular heaped irregular colony type form (Strains CF-1097-73, CF-470-72, CF-492-73, CF-99-72, CF-1098-73). Complete reversion from smooth to rough morphology was seen with Strains CF-492-73 and CF-99-72 (Figures 4a and 4b). Other strains occasionally showed a partial change in morphology; however, this did not persist in subculture. Smooth colonies from broth and agar cultures microscopically demonstrated a predominance of diphtheroidal bacilli-type forms with rare or no filaments (Figure 5). Diphtheroids were arranged in X, Y, V and S shapes, occasionally forming knobs and clubs in older cultures. No microcolonies were seen in these forms. The broth and agar cultures of granular colonies formed spidery microcolonies which microscopically consisted of a mass of tightly interwoven filaments and diphtheroids (Figures 6a and 6b). Branches, X, Y, V, and S forms were common.



Figure 4a. CF-99-72. Reversion from rough to smooth colonial morphology. 2X.



Figure 4b. CF-99-72. Reversion from rough to smooth colonial morphology. 2X.

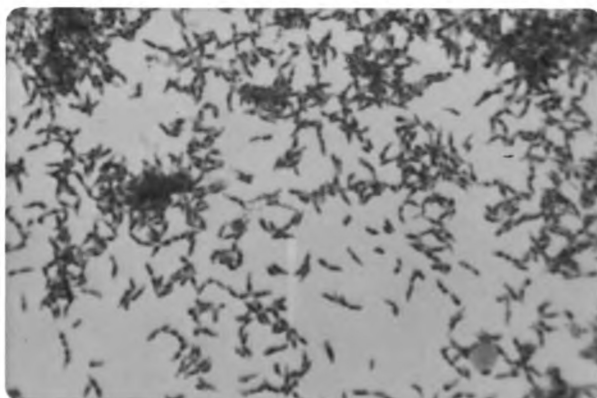


Figure 5. CF-194-74. Diphtheroid form of *A. viscosus* associated with smooth colonial morphology. Gram's stain; X 350.

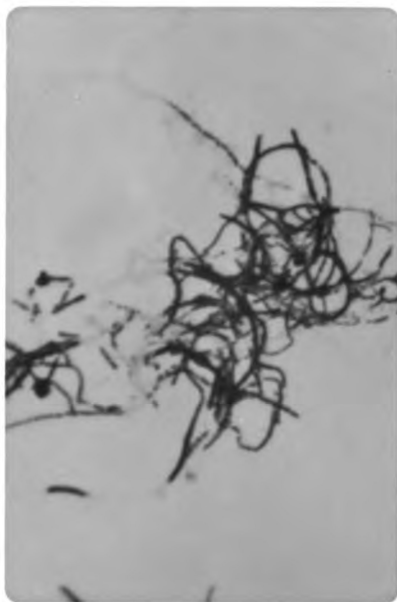


Figure 6a

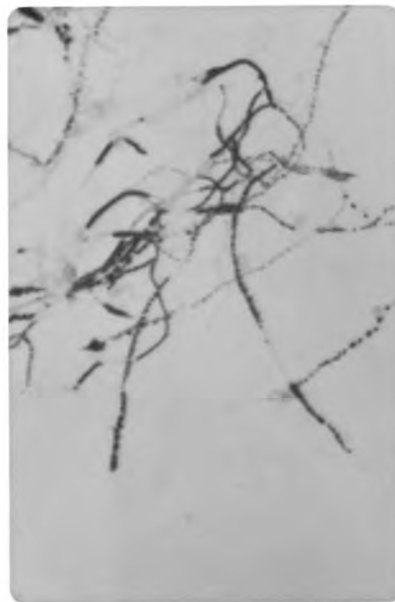


Figure 6b

Figures 6a and 6b. CF-470-72. Filamentous form of *A. viscosus* associated with rough colonial morphology. Gram's stain; X 350.

Knobs, clubs and highly irregularly-shaped filaments became more frequent in older cultures. Beading was often seen in Gram-stained and acid-fast-stained smears when long thin filaments were examined. Filaments were shorter, diphtheroidal and coccobacilliforms were more frequent, and pleomorphic shapes, knobs, and clubs were rare. Broth cultures associated with smooth colony formation showed ropy growth while broth cultures from rough colony forming strains demonstrated a loose flocculent, granular type of growth. Anaerobically prepared media with 10% carbon dioxide showed slight or no differences in amount of growth when compared with aerobic cultures; however, studies of broth cultures prepared with pure nitrogen demonstrated significantly less growth. All pure anaerobic cultures demonstrated very weak growth, but total inhibition was not evidenced.

Biochemical Tests

All organisms were catalase positive, indol negative, gelatinase negative, hydrolyzed aesculin, hydrolyzed starch, reduced nitrates to nitrites, urease negative, acetylmethylcarbinol negative, methyl red positive, and produced acid reaction in litmus milk (Table 3). The strains grew well on all carbohydrates. Carbohydrates fermented included fructose, galactose, glucose, lactose, starch and sucrose. Variable reactions were obtained from fermentation of glycerol, inulin, maltose mannitol, mannose melibiose, raffinose, ribose, salicin, sorbitol, trehalose, and xylose. Isolates did not ferment adonitol, arabinose, cellobiose, dulcitol, inositol, and rhamnose.

Table 3. Comparison of biochemical studies of *A. viscosus* strains isolated at MSU with reported reactions of *A. viscosus* and *A. naeslundii* cited in literature¹

Test	Strains									
	CF- 295- 73	CF- 470- 72	CF- 492- 73	CF- 99- 72	CF- 1078- 73	CF- 1097- 73	CF- 194- 74	<i>A.</i> <i>viscosus</i>	<i>A.</i> <i>naeslundii</i>	
Catalase	++	+	+	+	+	+	+	+	-	
Methyl red	+	+	+	+	+	+	ND	+	+	
Voges-Proskauer	-	-	-	-	-	-	-	-	-	
Nitrate red	+	+	+	+	+	+	+	+(-)	+	
Urease	-	-	-	-	-	-	-	-	-	
Gelatinase	-	-	-	-	-	-	-	-	-	
Aesculin hyd.	+	+	+	+	+	+	+	+(-)	+	
Starch hyd.	+	+	+	+	+	+	+	+(-)	-(+)	
Indole	-	-	-	-	-	-	-	-	-	
Litmus milk	acid	acid	acid	acid	acid	acid	acid	acid	acid	
Carbohydrate fermentations:										
Arabinose	-	-	-	-	-	-	-	-	-	
Adonitol	-	-	-	-	-	-	ND	-	-	

Table 3 (continued)

Test	Strains										A. <i>viscosus</i>	A. <i>naeslundii</i>
	CF- 295- 73	CF- 470- 72	CF- 492- 73	CF- 99- 72	CF- 1078- 73	CF- 1097- 73	CF- 194- 74					
Cellobiose	-	-	-	-	-	-	-	-	-(+)	-	-	
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	
Fructose	+	+	+	+	+	+	+	+	+	+	+	
Galactose	+	+	+	+	+	+	+	+	+(-)	-	-	
Glycerol	+	-	+	-	+	+	+	ND	V	-	-	
Glucose	+	+	+	+	+	+	+	+	+	+	+	
Inositol	-	-	-	-	-	-	-	ND	V	+	+	
Inulin	-	-	-	-	+	+	+	ND	V	V	V	
Lactose	+	+	+	+	+	+	+	+	+	+	+	
Maltose	-	+	+	+	+	+	+	+	+	+	+	
Mannitol	+	-	-	-	-	-	-	-	-	-	-	
Mannose	+	-	+	-	-	+	+	ND	+(-)	+	+	
Melibiose	-	+	-	+	-	-	-	ND	+(-)	+	+	
Raffinose	+	+	+	-	+	+	+	ND	+	+	+	

Table 3 (continued)

Test	Strain								A. <i>viscosus</i>	A. <i>naeslundii</i>
	CF- 295- 73	CF- 470- 72	CF- 492- 73	CF- 99- 72	CF- 1078- 73	CF- 1097- 73	CF- 194- 74			
Rhamnose	-	-	-	-	-	-	-	-	-	-
Ribose	-	-	-	-	+	-	+	+	-	-(w)
Salicin	-	+	+	+	+	+	ND	+	+	+
Sorbitol	-	-	-	-	+	-	-	-	-	+
Starch	+	+	+	+	+	+	+	+	+	V
Sucrose	+	+	+	+	+	+	+	+	+	+
Xylose	-	-	-	+	-	-	-	-	-	-
Trehalose	+	+	+	-	+	+	+	+	V	V

¹ Variations in experimental techniques and strains examined in different laboratories have led to much confusion in choosing the standard expected reactions to *A. viscosus* and *A. naeslundii*. The reported reactions of *A. viscosus* and *A. naeslundii* are composites of results from several studies (Howell *et al.*, 1963; Howell *et al.*, 1965; Slack *et al.*, 1968; Gerencser *et al.*, 1969; Gledhill *et al.*, 1969; Georg *et al.*, 1972; Holdeman *et al.*, 1972; W. E. C. Moore, personal communication).

* + = positive reaction; - = negative reaction; ND = test not performed; V = variable; w = weak.

DISCUSSION

Three questions must be considered when evaluating the clinical importance of *A. viscosus*:

1. Is it possible to differentiate *A. viscosus* from other microorganisms?
2. Is accurate speciation of this microorganism of clinical importance to the microbiologist?
3. Does isolation of *A. viscosus* from clinical lesions signify infection in the host?

Numerical taxonomy and monothetic Darwinian taxonomy have been analyzed in differentiation of *A. viscosus* from other filamentous and diphtheroidal microorganisms. Numerical taxonomy is a successful method for grouping distinctly different genera, but speciation and separation of biochemically and morphologically related genera require consideration of other factors. *Corynebacterium*, *Rothia*, *Bacterionema*, *Actinomyces*, *Nocardia*, *Arachnia*, and *Bifidiobacteria* morphologically resemble each other. *Actinomyces viscosus* produces a diphtheroidal form which cannot be distinguished from *Corynebacterium* and a filamentous form which resembles these other genera. Acid-fast stains are not of much use unless simultaneous controls are included. Inadequate decolorization will produce false positive results, and excessive use of acid in decolorizing can give false negative reactions. Some species of

corynebacteria may be partially acid-fast when stained by the Kinyoun's modified acid-fast stain (Harrington *et al.*, 1966). Certain species of *Nocardia*, and old cultures or excessively subcultured specimens of *N. asteroides* may stain non-acid-fast. Biochemical characterization will aid in speciation of most of the microorganisms, but certain problems in technique may be encountered. Review of literature concerning biochemical identification of *A. viscosus* has shown significant differences in results. Choice of media, size of inoculum, length of incubation, method of analysis, and number of strains examined are the probable causes of discrepancies. Slow-growing, fastidious microorganisms require longer periods of incubation and enriched media, which are not used in many clinical laboratories. Biochemical tests suggested in Appendix A, performed by described procedures, should be adequate for differentiation of *A. viscosus* from similar microorganisms.

Actinomyces viscosus strain CF-470-72 was sent to L. K. Georg at CDC; however, the organism was not viable when it arrived in Georgia. A catalase test and fluorescent antibody studies were still performed. Georg reported a negative catalase reaction, yet the strain was clearly catalase positive when it was examined at the MSU Clinical Laboratory. The catalase enzyme probably had been degraded when the strain died, thus producing the negative result.

The VPI method of examining the change in pH of carbohydrate test media is a sensitive technique for determination of fermentation of sugars. Holdeman *et al.* (1972) reported weak fermentation of xylose, and negative to weak fermentation of ribose by *A. viscosus*. Strains CF-1078-73 and CF-194-74 fermented ribose and Strain CF-99-72 fermented

xylose. Many carbohydrates are not fermented by all strains of *A. viscosus*; thus, only the recommended biochemical tests suggested in Appendix A should be used as criteria for rapid speciation.

Cell wall composition is a good analytical technique for separation of diphtheroids. This requires complicated apparatuses, gas chromatography or extensive extractions and chemical modifications which are not available to most clinical microbiology laboratories. Examination of cell walls for presence of diaminopimelic acid, lysine, mannose, ornithine, deoxytalose, and fucose is the minimum procedure for speciation of *A. viscosus* by this method.

Serology is a popular taxonomic procedure used in many specialized large diagnostic laboratories. Agglutination studies are of limited value; however, fluorescent antibody and immunodiffusion tests are quite sensitive for differentiation of *A. viscosus* from most other pleomorphic Gram-positive microorganisms. Unadsorbed fluorescent antibody which will react with all strains of *A. viscosus* and shows no fluorescent reaction with *A. naeslundii* is not presently available. Three strains of *A. viscosus* isolated from canine infections at the Clinical Microbiology Laboratory at Michigan State University did not fluoresce with the fluorescent antisera prepared at CDC. M. A. Gerencser at West Virginia University has reported that many strains she isolated did not fluoresce with these antisera. Presently the MSU isolates are being examined by her. Hopefully, the fluorescent antibody she has prepared will be more sensitive.

Gas-liquid chromatography is an expensive but necessary and rapid technique for speciation of anaerobic bacteria (Holdeman *et al* , 1972)

and may be useful in separation of genera of diphtheroids. Analysis of end-products from fermentation of glucose has produced a separate group of the actinomycetes. Speciation of *A. naeslundii* and *A. viscosus* with this technique has not been reported, but quantitative measurements of the amount of acetic acid produced when cultures are grown aerobically without additional carbon dioxide may be of value. *Actinomyces viscosus* reportedly grows better in oxygen when carbon dioxide is added, while *A. naeslundii* apparently shows no requirement for CO₂ in aerobic cultures. Quantitative analysis of end-products may be lower with *A. viscosus* than with *A. naeslundii* when grown in pure oxygen environment.

Electron microscopy is of doubtful use in taxonomy of diphtheroids at the present time. It may be of some value in the future, but cost will probably not allow it to be a tool for bacteria or fungal speciation in a clinical laboratory.

Correct speciation of diphtheroids is necessary when the microorganism is isolated in pure culture from a clinical specimen. *Actinomyces viscosus*, when freshly isolated from suppurative material, may form either palisading pleomorphic Gram-positive rods similar to *Corynebacterium* sp., or filaments closely resembling *N. asteroides* and other members of the Actinomycetales. Colonial morphology may be smooth, entire, mucoid, glistening, and convex or rough, heaped, granular, irregular, and dry. Both forms may be pathogenic. Routine biochemical media with the addition of PPLO-serum (Difco) reduces laboratory cost, yet is adequate for speciation. Infections from actinomycetes, *Nocardia* and corynebacteria are not common; however,

they are being reported. *Nocardia* and certain *Actinomyces* infections cannot be differentiated without cultural identification since the lesions of both types of infections may be closely similar. It is essential that the cause of subcutaneous granulomas be correctly determined as soon as possible so that proper antibiotic treatment can be instituted. Once draining sinuses and fistulous tracts have formed or progressive empyema has developed, the prognosis is poor in spite of antibiotic therapy and surgical excision. *Nocardia* sp. are more virulent and the infections are treated with different drugs. Penicillin is of little use in *Nocardia* infections, yet most *Actinomyces* strains are extremely sensitive to this drug. A report of a "diphtheroid, probably contaminant" in cases of chronic granulomatous and suppurative infections may serve as a tremendous hindrance to effecting a cure for the patient.

One must carefully evaluate reported references to diphtheroidal species in the literature. Carbon dioxide requirements for aerobic growth were not clearly evidenced with the canine strains of *A. viscosus* examined by Gerencser (Gerencser *et al.*, 1969) and in this study, yet others use this as one of the definitive characteristics of *A. viscosus* (Georg *et al.*, 1972). Reports that *A. naeslundii* does not ferment starch, and many erroneous generalizations about lactose fermentation and other biochemical reactions of *Corynebacterium* have been made (Holmberg *et al.*, 1972; Georg *et al.*, 1969a). Morphological variation within the same strain of diphtheroidal microorganisms is common; presence of two or three different colonial types on an agar medium, or presence of diphtheroidal and filamentous microorganisms in a Gram stain, may not necessarily indicate contamination.

Actinomyces viscosus, other actinomycetes, and diphtheroids are part of the normal flora in periodontal plaque of man, hamsters and probably other animals. Isolation of these microorganisms from plaque or sputum does not usually indicate infection. Multiple isolation of a diphtheroid in pure culture, or presence of filaments in granules isolated from granulomatous or suppurative tissue, is necessary to confirm infection with *A. viscosus*. When filamentous microorganisms are observed in granules, even if smaller amounts of bacteria are present, the former microorganism is the probable cause of infection.

Swerczak *et al.* (1968) has indicated that dogs under one year of age are more susceptible to *Nocardia* infections, while dogs older than one year are more susceptible to *Actinomyces* infections. Both cases of canine actinomycosis due to *A. viscosus* reported by Georg *et al.* (1972) occurred in dogs less than six months of age. Attempts to correlate size of dog and environment of the animal to *A. viscosus* infections have been made. Hunting dogs and other large breeds most likely are exposed to major and minor traumas such as puncture wounds, abrasions, and gunshot wounds which become contaminated with soil bacteria causing mild debilitation. This lowering of body defenses is apparently necessary for endogenous organisms to proliferate. *Actinomyces viscosus* is most likely a secondary invader superimposed on an underlying disorder. Four of the five cases described in this thesis were found in dogs with histories of previous disease which may have caused temporary debilitation (gunshot wounds, epilepsy, osteochondritis of the joints). These findings have not been examined in other reports, and the actual significance of minor debilitation is only a theoretical

surmise. *Actinomyces viscosus* is not as pathogenic for healthy laboratory animals as *N. asteroides* or other actinomycetes. However, it occasionally infects dogs, mostly large breeds, and rarely infects man and cats. Signs of chronic granulomatous tumorlike swellings, draining abscesses leading from fistulous tracts, and dyspnea, emaciation and low grade fever seen with thoracic empyemas are frequent observations in actinomycosis and nocardiosis. Hard, well defined actinomycotic granules result from deposition of acidophilic material around filaments. Granules associated with nocardiosis and *A. viscosus* are soft or white, flaky, poorly defined masses of filaments which are not cemented with this hard calcium-phosphate matrix.

Purulent material and granules have been occasionally submitted to the microbiology laboratory for culture and yet no growth on media was observed. Direct smears may have shown Gram-positive branching filaments, and specimens had been immediately cultured aerobically and anaerobically with little success. No explanation has satisfactorily accounted for all instances of failure to grow *A. viscosus* or other filaments. Occasionally inadequate specimens are submitted to the laboratory, or time between collection of specimen and culturing has been prolonged, which can kill fastidious pathogens. Bacteria present in granules may rapidly overgrow fastidious filaments on routine media. A final contributing cause for poor isolation of *A. viscosus* is recent antibiotic therapy, which inhibits growth or kills most of the microorganisms. The best specimen for submission to the laboratory is immediate delivery of granules obtained from the infected area before antibiotic treatment has begun.

ADDENDUM

Clinic Case 147495 (CF-194-74)

After completion of most of the research for this thesis, another case of canine actinomycosis was diagnosed at the Clinic. Lacking sufficient data to be included in the body of this thesis, morphological and biochemical properties of the strain are presented in an abbreviated form.

A 1.5 cm-sized swelling was noticed in the cervical region of a six-year-old female Norwegian Elkhound late in January 1974. The family veterinarian prescribed penicillin and streptomycin, which apparently caused temporary regression of the mass. No history of hunting accidents had been reported; however, the dog reportedly spent a lot of time outdoors and in wooded areas. The mass increased in size to about 6 cm in diameter by March 4, 1974, so the veterinarian referred the case to the Michigan State University Veterinary Clinic. The abscess was excised and drained, and portions of tissue and exudate were collected for examination. The animal was treated with penicillin and streptomycin and was discharged on March 7. A month later, the animal was reported in excellent condition, with no signs of regression.

Examination of a smear from the aspirated exudate did not reveal granules or filamentous microorganisms. A large number of nucleated cells, $248,000/\text{mm}^3$, neutrophils, erythrocytes with occasional

eosinophils, histiocytes, and mononuclear phagocytes were observed. Histological examination of the tissue revealed a chronic suppurative granuloma consisting of highly fibrotic connective tissue and vascular proliferative tissue infiltrated with neutrophils, histiocytes, and plasma cells. The granuloma penetrated surrounding skeletal muscle causing some myodegeneration. Gram stains of this specimen (Figure 7) showed Gram-positive branching filamentous microorganisms in discrete masses without any club-shaped borders, which is suggestive of *A. viscosus* or *Nocardia*. Confirmation of the diagnosis was made from isolation and identification of the etiologic agent, *A. viscosus*.

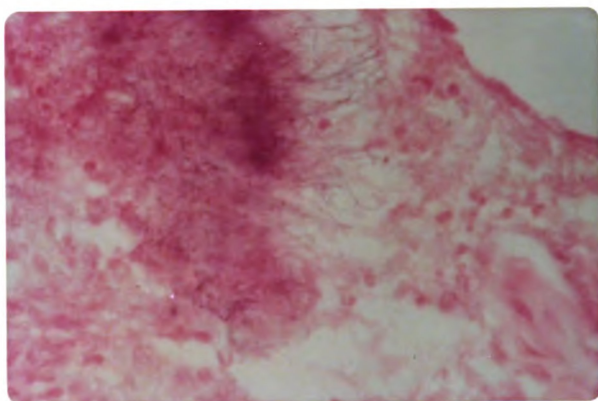


Figure 7. CF-194-74. Histopathology of *A. viscosus* granules from cervical abscess. Hematoxylin and eosin stain; X 1350.

This strain grew slowly on blood agar, did not grow on Sabouraud's agar, but grew well on supplemented BHI-blood agar under anaerobic and aerobic conditions with increased carbon dioxide. Broth cultures revealed both rope-like and flocculent growth. The colonial morphology was of the smooth form with a slight tendency to form mucoid, heaped colonies. Gram stains of a 48-hour agar culture showed short filaments and diphtheroidal elements. Branching was infrequent, but a predominance of X and V forms was apparent. Biochemical reactions of this non-acid-fast, catalase-positive strain, as reported in Table 3, are typical of *A. viscosus*.

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APPENDIX

APPENDIX A

TECHNIQUES FOR ISOLATION AND IDENTIFICATION OF A. *VISCOSUS* IN THE CLINICAL LABORATORY

Actinomyces viscosus can be identified on routine media in the clinical laboratory. This organism must be differentiated from anaerobic *Actinomyces* species, *Nocardia* species, and diphtheroids, since similar lesions have been reported with infections from these organisms (Georg *et al.*, 1972; Johnson *et al.*, 1970; Kalnins, 1963; Bruner *et al.*, 1973). Granules are the best sources of the etiologic agent; however, purulent exudate may be adequate if no granules are present. Granules should be washed with physiological saline and crushed with sterile glass rods. Some material should be saved for Gram stains and acid-fast stains. The crushed granules should then be cultured aerobically and anaerobically on supplemented BHI agar with 5% bovine blood (Holdeman *et al.*, 1972); however, trypticase blood agar will suffice if this is not available. Sabouraud's agar should also be inoculated under aerobic conditions. BBL-anaerobic jars with GasPak disposable H₂-CO₂ generator envelopes will provide nitrogen and carbon dioxide for maximal anaerobic growth. Aerobic cultures should be placed in a carbon dioxide incubator or candle jar if possible.

Anaerobic actinomycetes will grow on routine prereduced media such as chopped meat glucose broth (Robins Laboratories) and on prereduced

anaerobically sterilized culture media (PRAS). Thioglycollate broth and media capped with pyrogalllic acid seals have been reported to be toxic for many anaerobes (McMinn *et al.*, 1970). Actinomyces Maintenance Media (BBL) (Pine *et al.*, 1959) is an excellent medium. However, it is not available in most clinical laboratories.

A modified acid-fast stain by Kinyoun (Georg *et al.*, 1961) is more sensitive to partially acid-fast organisms such as *Nocardia* species than the Ziehl-Neelsen stain, but it is essential that controls be stained along with the specimen unless the microbiologist is confident of his techniques. Some *Nocardia* species can be non-acid-fast and occasional strains of *Corynebacterium* are partially acid-fast; thus further biochemical tests are necessary.

Once good growth on media is achieved, catalase production, colonial morphology, and Gram stains should be examined. Presence of growth on Sabouraud's agar excludes the possibility of *Actinomyces* species. Although biochemical media with yeast extract are preferred, routine biochemical test media supplemented with two drops of PPLO Serum Fraction may be substituted. These added factors encourage rapid growth of fastidious or slow-growing microorganisms. Adequate differentiation from *Lactobacillus*, *Rothia*, *Erysipelothrix*, *Actinomyces* species other than *A. viscosus*, *Bacterionema*, *Corynebacterium*, *Arachnia* and *Bifidiobacterium* (Table A-1) can be effected if the following biochemical media are inoculated: urea, gelatin, aesculin, Voges-Proskauer, lactose, and nitrate. Heavy inoculum from 48-hour cultures is recommended, and biochemical media should be incubated at 37 C in a candle jar or BBL GasPak A-jar for a minimum of seven days. Caution should be

Table A-1. Expected reactions of various Gram-positive filamentous or diphtheroidal microorganisms¹

Organisms	Tests ²									
	Oxygen Requirements	Growth on Sabouraud's Agar	Acid-fast	Catalase	Urease	Nitrate Reduction	Gelatinase	Lactose	Voges-Proskauer	Aesculin Hydrolysis
<i>Nocardia</i>	Aerobic	+	partially	+	V	-	V	-	-	ND
<i>Bifidobacterium</i>	Anaerobic	ND	-	-	-	-	-	+	V	ND
<i>Bacterionema</i>	Aerobic, facultative	ND	-	+	-	+	-	-	+	+
<i>Anachnia</i>	Anaerobic	ND	-	-	-	+	-	+	-	-
<i>Corynebacterium</i> 3	Varies	ND	-(w)	+	V	V	V	-(+)	-(de-layed)	-
<i>Erysipelothrix</i>	Aerobic	ND	-	-	-	-	-	+	ND	ND
<i>Lactobacillus</i>	Varies	ND	-	-	-	V	-	-	-	ND
<i>Rothia</i>	Aerobic, facultative	ND	-	+	-	ND	-	-	+	+
<i>Actinomyces</i>	Varies, usually	-	-	-	-	V	-	+	-	+
(not <i>A. viscosus</i>)	anaerobic	-	-	-	-	-	-	-	-	-
<i>A. viscosus</i>	Facultative	-	-	+	-	+	-	+	-	+

¹Holmberg *et al.*, 1972; McAllister, 1970; Holdeman *et al.*, 1972; Laskin *et al.*, 1973; Gledhill *et al.*, 1969; Carter, 1973.

²ND = not determined; - = negative reaction; + = positive reaction; V = variable reaction; w = weak reaction.

³Nonhemolytic *Corynebacterium* species typically produce the following reactions: catalase +, nitrate +, lactose +, urea -, gelatin -.

taken to prevent contamination since the media are incubated for such long periods of time. Routine laboratory tests for determination of biochemical reactions are satisfactory.

Actinomyces viscosus may or may not form filaments in culture, is non-acid-fast, catalase positive, hydrolyzes aesculin, ferments lactose and usually reduces nitrates. Urease production and Voges-Proskauer reactions are negative. Smooth or rough colonial morphology is evident on aerobic and anaerobic culture media. No growth occurs on Sabouraud's agar (Table A-1).

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