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A Bacteriological Study of Abscesses of Swine and Cattle

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A BACTERIOLOGICAL STUDY OF ABSCESSES OF SWINE AND CATTLE

BY

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A BACTERIOLOGICAL STUDY OF ABSCESSES OF SWINE AND CATTLE

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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"Men work together," I told him from the heart,

"Whether they work together or apart."

LJB

INTRODUCTION

Condemnation of abscessed portions of swine and beef carcasses is an important economic problem to the meat packing industry and, consequently, to the individual stock producer.

The Meat Inspection Division of the Agricultural Research Service of the U. S. Department of Agriculture, in its summary of activities from 1955 through 1965 (2), reported that the numbers of abscessed portions from cattle and swine have increased (Table 1 and Figure 1). In this 10-year period the number of abscessed parts condemned in swine increased from 1.4% (1,397,248) of the animals slaughtered to 3.9% (2,660,522). During the same period of time the number of abscessed beef livers condemned increased from 7.6% (1,432,505) to 8.8% (2,242,147), an increase of 1.2%.

The entire head of a hog is condemned when it has a jowl abscess and the entire liver of a beef is condemned when it is abscessed. The cost to the industry is currently $1.16 per hog head and $1.40 per liver (37). Using the above condemnation figures, the cost to the industry in 1965 was $1,620,807.68 for swine jowl abscesses and $3,139,005.80 for beef liver abscesses.

Although the percentage of abscessed beef heads which were condemned (Table 1 and Figure 1) declined from 1.6% in 1955 to 0.75% in 1965, the condemnations still cost the industry $754,842 in 1965.

In addition to the loss from abscesses which can be calculated directly from packing house losses, the individual stock producer loses by forced premature sale of breeding stock and by reduced feed

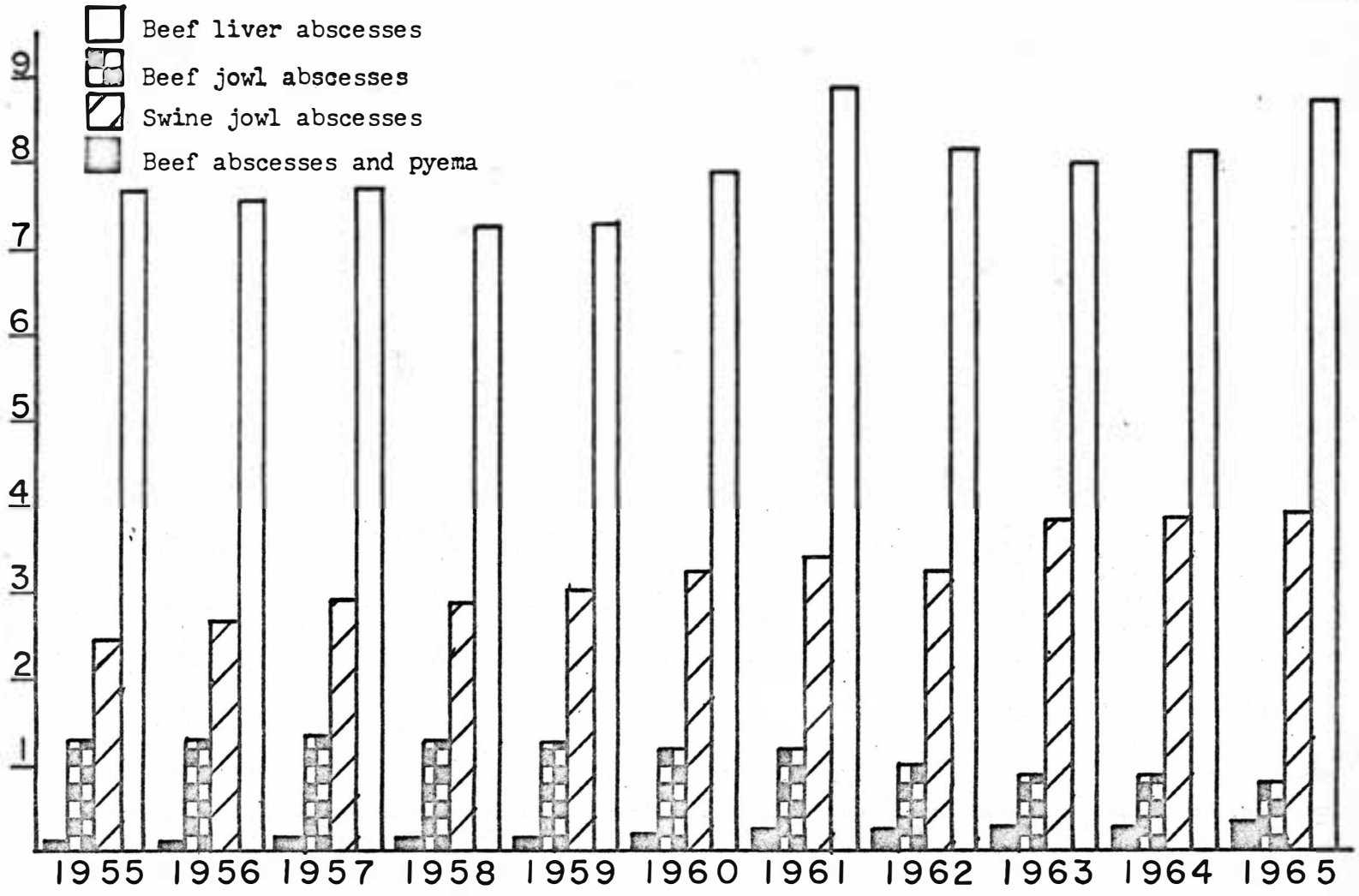

Figure 1. Percent of total animals slaughtered with condemned abscessed parts (calculated from Table 1)

utilization by an infected animal. The total effect on the industry is in the millions of dollars.

Workers in this field have indicated that bacteria are closely associated with abscesses in livestock, but additional information is necessary if this condition is to be alleviated. Therefore, this study was initiated to determine:

1) if the use of anaerobic techniques would yield any new information about the bacterial etiology of abscesses which had previously been studied using aerobic techniques.

2) if the rise in incidence of beef liver and swine jowl abscesses is due to an increase in previously reported organisms or to a new etiological agent.

3) the etiological agent of swine udder abscesses in the United States as no survey of the etiology of this condition has been made in this country.

4) if Actinomyces suis, isolated from swine udder abscesses in East Germany, is present in similar lesions in swine in the United States. If an organism is found which is similar to A. suis, it will be compared, biochemically and serologically, with known Actinomyces sp. and similar organisms.

5) if there has been any change in the etiology of beef jowl abscesses.

6) if abscesses from other organs from beef and swine have the same etiological agents as the abscesses in beef livers, beef jowls, swine jowls, and swine udders.

LITERATURE REVIEW

The Etiology of Beef Liver Abscesses

Beef livers are condemned because of four principal pathological conditions: liver fluke infestation, telangiestasis, "sawdust liver," and liver abscesses. The liver fluke infestation is beyond the scope of this study as the agents of this disease are parasitic worms. Telangiestasis is a condition of liver characterized by single or multiple dark red foci throughout the parenchyma, resulting from vascular congestion and hemorrhage in the foci. "Sawdust liver" is a meat inspector's term and refers to a condition of livers characterized by single or multiple gray colored foci, 1 to 2 mm in diameter. Microorganisms are seldom of significance in these two types of liver conditions.

The condition responsible for the greatest number of condemnations is the abscessed liver. One of the earliest reports of bovine liver abscesses is found in the treatise by Bang (as quoted by Madin, 52) in which he described a form of liver abscess obtained from slaughter house cattle and from which he was able to culture an organism he called "necrosis bacillus."

Bang's classic description of the condition is quoted as it accurately describes the gross pathology as seen today:

> The disease is usually found in healthy appearing animals at slaughter. The liver is more or less enlarged, and contains as a rule a greater or lesser number of walnut to egg-sized abscesses, which lie partially in the depths of the organ, and partially on the surface prominences. The abscesses are surrounded by a thick (externally fibrous, internally soft) capsule, and contains a thick tenacious pus.

> Embedded in this, one finds on closer examination solid clumps similar in size to the necrotic areas previously described. If one cuts through such a clump one sees that it consists of homogeneous dry, grey, necrotic tissue. On the outside of these clubs, and in the pus I have found the necrosis bacillus; through inoculation the identity of the organism occurring in liver necrosis was established.

Mohler and Morse (57) in 1900 isolated an organism from the liver of a deer and from bovine livers which they called Bacillus necrophorus. Subsequently additional investigators confirmed their findings and B. necrophorus was accepted in early standard veterinary texts as the etiological agent of liver abscesses. This is the same organism later named Sphaerophorus necrophorus by Prevot and Raynaud (66). The latter nomenclature is currently used by Breed et al. (9) in the 7th Edition of Bergey's Manual of Determinative Bacteriology. Madin (52) isolated Sphaerophorus necrophorus in pure culture from 85% of the abscessed livers of fat beef cattle and in mixed culture from an additional 10% of the abscesses.

Newsom (59) indicated that liver abscesses were associated with the feeding of beet by-products to cattle. He postulated that organisms gained entrance through abrasions in the digestive tract and were carried to the liver by the portal system. Dack et al. (17) added support to this theory by showing that the intestinal environment is favorable for the organism.

Jensen et al. (43) suggested that telangiestasis predisposes the liver to abscesses. The hemorrhagic condition of telangiestasis is transformed to "sawdust liver" by hemolysis of erythrocytes and the

infiltration of leucocytes. The dead tissue present in these conditions constituted a favorable condition for the invasion of S. necrophorus.

Jensen et al. (41, 43) were of the opinion that S. necrophorus entered the liver through the portal blood stream via lesions in the rumen. They stated, "In fattened beef cattle, rumenitis occurs commonly. The lesions probably lower efficiency of utilization of feed. Through foci of injury, bacteria, especially S. necrophorus, are able to penetrate the rumen wall, enter the portal blood, and be carried to the liver where secondary foci of infection may become established."

Later, Jensen et al. (42) inoculated cattle via the intraportal route with S. necrophorus and produced liver abscesses. The abscess formation began eight days after inoculation and reached maximum size by 30 days. The central necrotic liver tissue persisted for as long as 100 days.

The theory that S. necrophorus invaded the liver through the rumen wall gained support when Robinson et al. (68) successfully isolated S. necrophorus from the rumen.

Hagan and Bruner (33) postulated that "this organism has little or no ability to invade normal mucous membranes of the skin but it thrives in wounds of the surface produced by mechanical injury or bacterial action." However, Hagan and Bruner (33) and Canada (10) reported the production of a soluble exotoxin and a soluble, heat-stable, chemical-resistant endotoxin. When injected intraperitoneally in mice, the exotoxin produced no effect. Merchant and Packer (55) reported that the exotoxin produced edema when injected intradermally in rabbits. He

was of the opinion that the production of a necrotizing endotoxin by the organism is a contributing factor to its ability to produce lesions in animal tissue.

The following organisms have been reported in mixed infections with S. necrophorus or in pure culture in a small percentage of abscessed livers but appear to be of minor importance: Corynebacterium pyogenes, Micrococci, Pseudomonas aeruginosa, and Escherichia coli (27).

The Etiology of Beef Jowl Abscesses

The beef jowl abscess, commonly known as lumpy jaw, was first described by Bollinger in 1877 (6). Harz (34) described the causative agent as Actinomyces bovis and actinomycosis became a common synonym for the condition. Lignieres and Spitz (50, 51) in reports of their investigations of actinomycosis of cattle in Argentina differentiated between two types of jaw lesions. One, an infection of the bone tissue, was caused by a Gram positive fungus-like organism (A. bovis) and the other, an infection of the soft tissue, was caused by a Gram negative diplobacillus which they named Actinobacillus lignieresii.

Griffith (31) confirmed that actinomycosis of the lymph glands of the head, lips, tongue, and buccal mucosa yield only a Gram negative diplobacillus which was similar in morphological, cultural, and biochemical characteristics to the organism originally described by Lignieres and Spitz. In actinomycosis of the jaw bone, Griffith found a filamentous Gram positive organism. Gunst (32) in Holland, Bongert (7) in Germany, Hulphers (39) and Magnusson (53) in Sweden, Bosworth (8), Davies and Torrance (18) in England, and Higgin (36) in Canada further supported these observations.

Vowter (76) reported that, of 26 specimens of bone actinomycoses examined, 24 had club-bearing rosettes. Corynebacterium pyogenes was isolated from 7 of the lesions, Actinomyces bovis-like organisms from 10, and noncharacterized anaerobic diphtheroids from 4 of the 26 specimens.

Actinobacillus has been isolated from the ruminal contents of normal cattle (63). There is an antigenic relationship between ruminal isolates and pathogenic strains indicating that the source of the infection is endogenous. The Actinomyces also appear to come from an endogenous source as they have never been isolated other than from the host (27). Both types of infection have been associated with abnormal dental conditions which possibly provide points of entry for the causative organism. Entry could also be gained due to injury by feeds of the buccal or pharyngeal region.

The Etiology of Swine Jowl Abscesses

Abscesses of the pharyngeal region of hogs have been referred to as strangles of hogs, feeder boils, jowl abscesses, and cervical abscesses. This pathological condition was reported as early as 1898, in Romania, by Starcoricci (cited by Hutra *et al.*, 40). The disease was characterized in the advanced stage by the presence of one or more heavily encapsulated abscesses within the soft tissue of the ventral and/or lateral cervical region of the affected animal. The abscesses ranged in size from less than 1 cm to more than 10 cm in diameter. The smaller lesions were found embedded in the regional lymph nodes and the exudate contained within an abscess was characteristically odorless, distinctly greenish in color, and varied in consistency from creamy to gelatinous. Some of the superficial abscesses fistulated and drained through the skin. Superficially located abscesses were readily observable, but deep lesions were difficult to detect and were not always revealed until they were encountered on inspection of the affected region when the animals were slaughtered (13, 14).

In 1937, Newsom (58) isolated beta-hemolytic streptococci in pure culture from throat abscesses. Stafseth and Clinton (73) reported Group E streptococci in cervical abscesses of a 7-month-old pig. This pig was from a herd with a history of cervical abscesses. No clinical signs were observed but at necropsy 6 abscesses approximately the size of walnuts were found in the submaxillary region.

Snoeyenbos *et al.* (72) investigated a herd of 35 swine which, in 1949, had an incidence of about 40% infection. A similar morbidity rate for the same herd was reported in 1950 and 1951.

Collier (12) collected data on the incidence of jowl abscesses of swine located on a farm in northern Iowa. In 1951, 54 of 57 hogs were infected (95%) and in 1952, 284 of 300 (94.7%). A year later, even after complete herd reduction, disinfection of the swine unit, and restocking, the infectivity rate reached high levels within 3 months.

Collier (14) isolated the following organisms from 492 specimens collected at slaughter: 85.6% Group E streptococci, 1.42% Streptococcus equisimilis, 0.81% Streptococcus zooepidemicus, 3.8% Pasteurella multocida, 3.25% Proteus mirabilis, 1.07% Escherichia coli, 0.41% Actinomyces bovis, 0.20% Staphylococcus aureus.

Kohler (45), in Vienna, isolated C. pyogenes, E. coli, and S. aureus from swine jowl abscesses.

The Etiology of Swine Udder Abscesses

Organisms reported as the morbific agents of swine udder abscesses include: streptococci, staphylococci, Sphaerophorus necrophorus, Corynebacterium pyogenes, Mycobacterium tuberculosis, Actinobacillus lignieresii, and Actinomyces bovis (24, 40, 55, 56, 60). Adler (1), Hemholt (35), and Langham and Stockton (49) isolated Aerobacter aerogenes from the mammary glands and spleens of sows dying from acute mastitis.

Several researchers observed Actinomyces-like organisms in smears from sow mastitis, but could not isolate the organisms (7, 21, 67). Rader (67) found granules in 30 of 52 cases of chronic mastitis but was unable to culture the Actinomyces sp. aerobically.

Schenk (70) found granules in 15 of 19 infected udders and isolated anaerobic Actinomyces sp., in addition to other organisms, from 12 of these. Based on morphology, 19 isolates from these 12 infected udders were placed in 3 distinct groups.

Magnusson (53) examined 273 abscessed udders and made 181 isolations of anaerobic Actinomyces sp. He considered the etiological agent to be Staphylococcus sp. in 41 udders and Actinobacillus sp. in 9 udders. A rough variety of Actinomyces sp. was found in 99 cases, a smooth form in 71, and mixed infections with both colony types in 11 abscesses. Some strains of both colony types were serologically related, but they were distinct from A. bovis in their microscopic and serological characteristics.

It was the opinion of Englert et al. (23) that the major etiological agent of swine udder actinomycosis was still unknown. In

a later paper (1961) Englert (22) discussed the pathology and etiology of udder actinomycosis in swine and reported the isolation of a microaerophilic Actinomyces sp. from the lesions. He proposed the name Actinomyces suis for the causative agent of this disease. Grasser (29, 30) succeeded in cultivating two groups of actinomyces from udder infections in swine. Six isolates of one group were identified as A. israelii. Eleven isolates of the other group were reported as A. suis on the basis of biochemical reactions and the agglutination test.

MATERIALS AND METHODS

Collection of Abscessed Specimens

All samples were collected intact from the processing line on the "kill floor" of the Armour and Company slaughter house in Huron, South Dakota. The specimens were placed in individual plastic bags and stored in an ice chest for transport to the laboratory. The number of swine and beef processed was kept by a tally of the swine and beef slaughter chains. The number of heads condemned for jowl abscesses and the number of livers condemned for liver abscesses were tallied by the federal inspector responsible for that particular inspection.

Isolation of Bacteria

The samples were transported to the laboratory the same day in which they were collected. They were either processed that night or held in cold storage and processed the next morning. Each abscess was trimmed of all excess material, seared with a heated spatula, and opened with a sterile scalpel (69). Observations were made of the abscess size and the consistency, color, and odor of the exudate. A loopful of the material contained in the abscess was streaked directly on a plate of brain heart infusion (BHI) agar containing 5% defibrinated sheep blood. The plates were incubated for 5 days at 37 C. Anaerobiosis was maintained by placing the cultures in an anaerobic jar which had one

pound of moistened oats in the bottom. The atmosphere in the jar was evacuated and replaced three times with a 95% N_2-5% CO_2 gas mixture.*

After incubation, colonies were picked from each plate and inoculated into a fortified thioglycollate medium (TTST) which contained Bacto-fluid thioglycollate (24.0 gm), trypticase soy broth (1.5 gm), and tryptose broth (1.25 gm) in 1000 ml of water. These tubes were incubated anaerobically at 37 C for 5 days. Anaerobiosis, except as defined for initial isolation, was maintained by placing the cultures in an anaerobic jar in the presence of a Torbal catalyst** and an atmosphere of 80% N_2, 15% H_2, and 5% CO_2***.

Characterization of Bacterial Isolates

Gram stains (Hucker's modification, 54) of 5-day-old cultures in TTST were observed for purity and morphology. All slide preparations of the isolates also included smears of *E. coli* and *S. aureus* as a control on the Gram staining procedure.

Oxygen requirements for all organisms were determined using BHI slants which contained 5% sterile defibrinated sheep blood. One loopful of inoculum from a 5-day-old culture of each isolate grown in TTST was

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*The gas mixture was purchased from Ohio Chemical and Surgical Equipment Co., Cleveland, and was analyzed using gas chromotography to confirm the composition.

**A catalyst prepared by the Torsion Balance Co., Clifton, New Jersey, for use in their anaerobic containers to catalyze the reaction of hydrogen in the gas mixture added to the container with any free oxygen in the container.

***The gas mixture and an analysis of the composition were obtained from The Matheson Co., Inc., East Rutherford, N. J.

streaked on the surface of 3 slants. Each organism was incubated at 37 C in an aerobic, anaerobic, and microaerophilic (air + 10% CO_2) atmosphere. The results were recorded as follows for each culture:

3 + -- maximum growth

2 + -- moderate growth

1 + -- slight growth

0 -- no growth.

On the basis of Gram reaction, morphology, and oxygen requirements, the isolates were grouped as follows:

Gram Positive Organisms	Gram Negative Organisms
Rods: diphtheroids*	Facultative bacilli
bacilli**	Obligate, anaerobic bacilli
Cocci: staphylococci	
streptococci	

Staphylococci

Anaerobic Glucose Fermentation--To test for the anaerobic fermentation of glucose (74), inocula were prepared by growing the isolates for 24 hours at 37 C on tryptone yeast extract agar containing: Bacto-tryptone, 10.0 gm; Bacto-yeast extract, 0.1 gm; Bacto-agar, 15.0 gm; distilled water, 1000 ml. The fermentation test medium contained: Bacto-tryptone, 10.0 gm; Bacto-yeast extract, 1.0 gm; glucose, 10.0 gm; brom-cresol purple, 0.04 gm; Bacto-agar, 2.0 gm; distilled water, 1000 ml; adjusted to pH 7.0.

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*A term used to denote all of the Gram positive "diphtheria-like" organisms.

**The term bacilli denotes rod-shaped organisms.

Before use, the fermentation medium was steamed for 15 minutes and solidified by placing the tubes in an upright position in an ice bath. The tubes were immediately inoculated by stabbing to the bottom of the medium with a loopful of organisms. The surface of the tube was covered with a layer of sterile petrolatum 25 mm deep, and the tube was incubated 5 days at 37 C. An indicator change from purple to yellow throughout the medium was interpreted as the anaerobic production of acid from glucose.

Coagulase Test--To test for the presence of "free" coagulase (74) a single colony from a 24-hour blood agar plate was emulsified in 1.0 ml of fresh citrated rabbit plasma which had been diluted 1 to 5 with physiological saline. Known positive and negative controls were set up at the same time. The suspensions were incubated in a water bath at 37 C and examined for clotting of the plasma after 1, 2, 4, 8, and 24 hours. Any degree of clotting of the plasma was accepted as positive evidence of coagulase activity.

Streptococci

Precipitin Reaction--Extracts from the streptococcal isolates were tested against Bacto-Group E Streptococcus antiserum using the capillary tube precipitin test. Isolates which could not be grouped with the Group E antiserum were tested with the Group A Streptococcus antiserum. The following hot hydrochloric acid extraction method was used to prepare the soluble antigen (48).

1) The culture was inoculated into 40 ml of Bacto-Todd Hewitt Broth and incubated overnight at 37 C.

2) It was centrifuged at 3,500 rpm (International head 308) for 15 minutes and the supernatant was discarded.

3) 0.4 ml N/5 hydrochloric acid in 0.85% sodium chloride was added to the sediment.

4) A loopful of the suspension was placed in a drop of 0.01% thymol blue solution. The indicator turned orange indicating that the suspension was acidic.

5) The suspension was placed in a boiling water bath for 10 minutes.

6) The suspension was cooled and decanted to a clean tube containing a small drop of a 0.01% phenol red solution.

7) N/5 sodium hydroxide was added to pH 7.0-7.2 (orange).

8) The suspension was centrifuged and the supernatant extract was pipetted off.

A capillary tube 0.7-1 mm (i.d.) x 75-90 mm was inserted into the antiserum until a column of antiserum had risen 2-3 cm in the tube. The excess antiserum was wiped from the exterior of the tube and an equal amount of the prepared antigen (from step 8 above) was allowed to enter the tube. The column of antigen-antiserum mixture was allowed to move to the center of the tube and the tube was inserted in a plasticine block. Normal serum controls and Bacto-Streptococcus antigen controls were prepared. After 15 min the tubes were checked. If there was no precipitate in the normal serum control and precipitate was present with the known antigen control and test organism, the test was considered positive.

Gram Positive Bacilli

The bacilli were stained using the Wirtz method (54) to demonstrate whether endospores were present.

Aerobic Gram Negative Bacilli

The isolates were grown in TTST at 37 C and Gram stained at 12 hrs, 48 hrs, 72 hrs, and 5 days.

Photographs of the stained organisms were taken with a 35 mm Kodak model 2 camera through an American Optical microscope (XL10TG-QW), AO shutter assembly, and 5X adaptor. Kodachrome II colored film with a blue filter at the light source was used for colored pictures; Kodachrome plus X pan with a green filter at the light source was used for the black and white photographs.

Diphtheroids

Morphology

Gram Stains--Gram stains were prepared for comparative morphological study of organisms upon primary isolation and after several transfers. Photographs of representative organisms were taken using the microphotographic techniques previously described.

Microcolonies--Microcolonies were observed at 24 and 48 hours on the surface of BHI agar (10 ml/plate) incubated anaerobically at 37 C. A coverslip was carefully placed on the colonies and microscopic observations were made at 500 X and 1000 X. Photographs were taken through the AO microscope and shutter assembly combined with a Polaroid Land camera. Polaroid 3000 speed type 107 black and white film was used.

Macrocolonies--Macrocolonies were observed on both BHI agar plates and BHI agar plus 5% defibrinated rabbit blood. The plates were incubated anaerobically at 37 C for 7 and 14-day observations.

Biochemical Tests

Catalase Production--The organisms were tested for the production of catalase in both TTST and BHI media by adding several drops of 3% H_2O_2 directly to the medium which had been incubated anaerobically for 5 days at 37 C. The evolution of bubbles was considered a positive test for catalase. The cultures were also tested for catalase production on BHI slants which had been incubated aerobically, microaerophilically, and anaerobically for 14 days at 37 C. A loopful of the culture was removed from the slant, mixed with 1 drop of 3% H_2O_2 on a slide, and covered with a coverslip. The active production of bubbles was recorded as a positive test for the presence of catalase. Control tests were included for the medium, H_2O_2, slides, and coverslips.

Acid Fast Stain--The Ziehl-Neelson acid fast stain (54) was made on smears of 5-day-old cultures grown in TTST. A smear of Mycobacterium phlei was used as a positive control.

Hydrogen Sulfide Production--A slant of BHI agar was inoculated with a loopful of a 5-day-old culture of each isolate grown in TTST. A sterile lead acetate strip was inserted into each tube until the lower edge of the strip was just above the surface of the slant. Observations were made at 7 and 14 days. Blackening of the paper nearest the medium surface was considered a positive test. Lead acetate strips were prepared by cutting filter paper strips 5 x 50 mm and soaking them

in a saturated solution of lead acetate. The strips were placed in petri plates, autoclaved, and dried in an oven.

Starch Hydrolysis--Soluble starch and insoluble starch were used as substrates for hydrolysis determination. Five grams of starch were suspended in 40 ml of cold water, added to 1000 ml of nutrient agar and autoclaved. Fifteen ml of media were poured in each petri dish and streaked with a single line of inoculum. Three sets of the plates were incubated anaerobically at 37 C and checked for hydrolysis at 3, 7, and 14 days. The plates were flooded with 2-4 ml of Gram's iodine and the results observed immediately. A clear area along the streak was recorded as positive.

Litmus Milk--Tubes of litmus milk fortified with 0.5% yeast extract were inoculated with 3 drops of a 5-day-old culture grown on TTST. An uninoculated control was included and the tubes were incubated anaerobically at 37 C for 7 days. The tubes were returned to atmospheric conditions and allowed to stand until the control tube returned to its original color. The reactions were recorded as: pink--acid; purple--alkaline; pink with a solid curd--acid coagulation; semi-solid bluish curd--alkaline coagulation; digested curd with a fluid more viscous and turbid than whey--peptonization; white--reduction.

Fermentations--Fluid thioglycollate without dextrose or indicator reinforced with 2 gm yeast extract and 1 ml of 1% brom-thymol blue per liter was used as the base medium for fermentation reactions. Eight ml of basal medium was dispensed into 15 x 125 mm tubes, durham tubes were added, and the medium was autoclaved.

The carbon sources, with the exception of insoluble starch, were filter sterilized in 10% aqueous solutions with a Millipore filter (0.22 μ pore size). One ml of the sterile carbon compound to be tested was added to each tube of fermentation base.

Insoluble starch was prepared by adding 5 gm starch to 1000 ml thioglycollate base medium. The mixture was stirred continuously and dispensed in 10 ml aliquots per tube. A durham tube was added and the tubes were autoclaved. After addition of carbon compounds, sterility was checked by incubating all tubes at 37 C for 24 hours.

Inoculum was taken from 5-day-old growth in TTST medium and 3 drops of inoculum from a Pasteur pipette were added to each. The tubes were incubated anaerobically at 37 C.

Uninoculated tubes of the fermentation medium and tubes of inoculated basal medium with no carbon source were included as controls.

The tubes were observed at 7, 14, and 21 days for the production of acid and gas. A change from green to yellow was considered positive for production of acid. At 21 days those tubes which were equivocal were checked with a pH meter. A pH drop of 1 unit from the pH of the uninoculated control was considered positive for the production of acid.

Gelatin Hydrolysis--The medium for the gelatin test was prepared by soaking 4 gm of gelatin in 40 ml of cold water and mixing it with 15 gm of agar melted in 1000 ml of distilled water. The medium was autoclaved and dispensed in 15 ml aliquots into petri plates. Each plate was inoculated by placing one drop of culture on one side of the plate and streaking it across the plate in a single line. The plates were incubated anaerobically for 7 and 14 days and subsequently flooded

with 2-4 ml of bichloride of mercury solution (15 gm $HgCl_2$ + 20 ml conc. HCl + 100 ml distilled water). A test was positive for the hydrolysis of gelatin if there was a clear zone adjacent to the colony growth and a cloudy precipitate over the remainder of the medium surface.

Indole Production and Nitrate Reduction--Duplicate tubes of Indole-Nitrate broth were inoculated with each of the organisms and incubated anaerobically at 37 C for 3 and 7 days. Indole production was determined by adding and mixing 1 ml of xylene to each tube to concentrate the indole. The culture tubes were allowed to remain stationary for several minutes until the xylene had risen to the surface of the medium. Four to 5 drops of Ehrlich's reagent (1 gm para-dimethyl amino benzaldehyde, 95 ml 95% ethyl alcohol, 20 ml conc. HCl) were carefully added to the medium with the tube held in a slanted position. A red ring at the broth-xylene interface was considered a positive test. Positive and negative controls were included.

The reduction of nitrate was determined by adding 5 drops of sulfanilic acid reagent (8 gm sulfanilic acid in 1000 ml 5N acetic acid) followed by 5 drops of alpha-naphthylamine reagent (5 gm alpha-naphthylamine in 1000 ml 5N acetic acid) to the second set of Indole-Nitrate broth tubes. The development of a pink to red color was a positive test for the reduction of nitrate to nitrites. If the culture was negative, a small amount of zinc powder was added to determine if the nitrites had been further reduced. A resulting red color was considered a negative test for nitrate reduction. No change after the addition of zinc powder was recorded as a positive test for the

reduction of nitrate. This indicated that the reduction had gone beyond the nitrite state to either ammonia or free nitrogen. Negative and positive controls were included.

Serology

Antigens were prepared from each of the unknown diphtheroids and were tested against antisera of the organisms listed in Table 2.

Preparation of Antigens--The following methods of King and Meyer (44) were used to prepare the soluble antigens:

1) The cultures were inoculated into 6 dilution bottles each containing 100 ml of Actinomyces Maintenance Broth (BBL) plus 0.05% sodium thioglycollate and incubated at 37 C for 10 days.

2) Five-tenths ml of formalin was added to each of the culture bottles which was kept at room temperature overnight.

3) The cells were harvested by centrifugation (30 min at 3500 rpm International head No. 308).

4) The supernatant was cooled to 5 C and agitated with a magnetic stirrer while 2 volumes of acetone were slowly added, drop by drop. The precipitate was allowed to settle overnight.

5) The supernatant was removed and the precipitate washed twice with small volumes of acetone.

6) The precipitate was dehydrated and stored in a vacuum jar over silica gel.

7) The antigen was rehydrated for testing by preparing a 1:10 dilution with distilled water which contained merthiolate at a concentration of 1:10,000.

Table 2. Diphtheroid Cultures Used for Antiserum Production

Name	Number	Source
Actinomyces bovis	13683 ATCC	American Type Culture Collection (ATCC)
Actinomyces israelii	12102 ATCC	ATCC
Actinomyces naeslundii	12104 ATCC	ATCC
Actinomyces eriksonii	15423 ATCC	ATCC
Actinomyces odontolyticus	17929 ATCC	ATCC
Actinomyces parabifidus	17930 ATCC	ATCC
Actinomyces propionicus	14157 ATCC	ATCC
Actinomyces discofoliatus	19246 ATCC	ATCC
Odontomyces viscosus	15987 ATCC	ATCC
Nocardia dentocariosus	17931 ATCC	ATCC
Ramibacterium pleuriticum	19301 ATCC	ATCC
Unknown Diphtheroid	25	Swine udder
Unknown Diphtheroid	43	Swine udder
Unknown Diphtheroid	94	Swine udder
Unknown Diphtheroid	108	Swine udder
Unknown Diphtheroid	230	Swine udder
Unknown Diphtheroid	271	Swine udder
Unknown Diphtheroid	326	Swine udder
Unknown Diphtheroid	126	Beef liver

Preparation of Antisera--The antisera were produced by the following procedure:

1) The formalinized cells harvested in step 5 of the antigen preparation were washed with saline and suspended in 10 ml of saline.

2) The suspensions were homogenized with a Branson Sonifier for 1 min using a microtip and a power output setting of 3.

3) The homogenized suspensions were adjusted to an OD reading of 0.8 on the Bausch and Lomb spectrophotometer. This approximates a cell suspension density between No. 4 and No. 5 McFarland nephelometer tubes.

4) Pre-immunization serum samples were obtained by ear bleeding male albino rabbits.

5) Two rabbits were injected intravenously with each cell suspension prepared above using the following inoculation series:

Mon., Wed., Fri.,	-- First 3 weeks	0.5 ml
Mon.	-- Fourth week	1.0 ml
Wed.	-- Fourth week	1.5 ml
Fri	-- Fourth week	2.0 ml

6) The rabbits were test bled on the Friday of the fifth week and those whose serum gave a positive Ouchterlony test against their homologous antigen were exsanguinated.

7) The rabbits that had negative titers were again given the fourth week injection series and exsanguinated after one week.

Ouchterlony Agar Gel Diffusion Test--The agar-gel technique of Ouchterlony (61) as modified by Schubert et al. (71) was used:

1) The agar was prepared according to the following formula: 9.0 gm NaCl, 9.0 gm Noble agar, 4.0 gm sodium citrate, 1,000 ml distilled water.

2) The medium was autoclaved for 10 minutes and 2.50 ml phenol was added.

3) Ten ml quantities were dispensed into petri dishes and allowed to solidify.

4) Stainless steel cylinders were placed on the surface of the agar with 1 central cylinder and 6 surrounding cylinders 6 mm from the central well and an equidistance apart. Fifteen ml of the medium were added and allowed to cool. The steel cylinders were removed by a twisting and lifting motion.

5) The antigen was placed in the central well and the antisera were placed in the peripheral wells.

RESULTS AND DISCUSSIONS

Beef Liver Abscesses

During the periods of collection at the packing plant, 331 livers from 2848 cattle (or 11.8%) were abscessed. It is the opinion of the inspectors and workmen who have been slaughtering animals for many years that the numbers of abscessed livers are higher in feed-lot cattle than in cattle from other sources. Even in the relatively short period of time that these collections were made on the kill floor, it appeared that feed-lot animals had many more abscesses than stock cattle. The workmen and inspectors also pointed out that the infections were not evenly distributed throughout the feed-lot cattle. One lot of cattle which was processed during our collection period had 28 abscessed livers in a total of 79 head (38%), while other lots of similar numbers had no abscesses.

It is worthy of mention that in the lot of 79 cattle which had 28 abscessed livers, 23 of the remaining 51 had liver flukes. The importance of the liver fluke in liver abscesses requires further study. Possibly the flukes are carriers of the causative organisms or predispose the liver to infection by causing abnormal conditions.

The organisms most frequently isolated from the beef liver abscesses were Gram negative anaerobes (88.4%) which were assigned to the group bacteroides in this study. Of the 95 liver abscesses examined (Table 3), 71 (74.7%) contained pure cultures of bacteroides, 9 bacteroides in mixed culture with diphtheroids, and 4 bacteroides plus some organism other than diphtheroids.

Table 3. Isolations from Beef Liver Abscesses

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis
24	bacteroides	1.2 - 10.0	thick to mucoid	green	none	clear
10	bacteroides	1.2 - 7.6	thick to mucoid	green	none	none
12	bacteroides	1.2 - 2.5	thick to mucoid	tan	none	clear
1	bacteroides	2.5	thick to mucoid	tan	none	none
3	bacteroides	2.5 - 7.6	thick to mucoid	white	none	clear
4	bacteroides	1.2 - 5.0	thick to mucoid	white	none	none
2	bacteroides	2.5	thick to mucoid	yellow	none	clear
1	bacteroides	1.2	thick to mucoid	grey	none	clear
1	bacteroides	1.2	thick to mucoid	brown	none	clear
1	bacteroides	1.2	thick to mucoid	green	none	none
	bacteroides	1.2	thick to mucoid	green	none	clear
1	bacteroides	1.2	thick to mucoid	tan	none	clear
	bacteroides	1.2	thick to mucoid	tan	none	none
1	bacteroides	1.2	thick to mucoid	tan	none	clear
	bacteroides	1.2	thick to mucoid	tan	none	none
1	bacteroides	2.5	thick to mucoid	green	none	clear
	E. coli	2.5	thick to mucoid	green	none	none
1	bacteroides	2.5	thick	green	none	none
	Diphtheroid 258	2.5	thick	green	none	none

Table 3. (continued)

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis
1	bacteroides	2.5	thick	white	none	none
	Diphtheroid 289	2.5	thick	white	none	clear
1	bacteroides	3.7	thick	green	none	none
	streptococcus	3.7	thick	green	none	clear
1	bacteroides	1.2	thick	green	none	clear
	Bacillus	1.2	thick	green	none	none
1	bacteroides	7.6	thick	tan	none	none
	Bacillus	7.6	thick	tan	none	none
1	bacteroides	2.5	thick	green	none	none
	S. aureus	2.5	thick	green	none	clear
1	bacteroides	1.2	thick	green	none	none
	Diphtheroid 194	1.2	thick	green	none	none
1	bacteroides	2.5	thick	green	none	clear
	Diphtheroid 217	2.5	thick	green	none	none
	Diphtheroid 218	2.5	thick	green	none	none
1	bacteroides	1.2	thick	white	none	clear
	bacteroides	1.2	thick	white	none	none
	Diphtheroid 260	1.2	thick	white	none	clear
1	bacteroides	2.5	thick	white	none	clear
	Diphtheroid 122	2.5	thick	white	none	none

Table 3. (continued)

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis
1	bacteroides	10.0	thick	green	none	clear
	Micrococcus	10.0	thick	green	none	none
	Diphtheroid 117	10.0	thick	green	none	clear
1	Diphtheroid 79	2.5	thick	white	none	clear
	Diphtheroid 80	2.5	thick	white	none	none
1	Diphtheroid 252	1.2	watery-bloody	bloody	strong	none
	Diphtheroid 253	1.2	watery-bloody	bloody	strong	none
1	Diphtheroid 287	1.2	thick	green	none	clear
	Diphtheroid 288	1.2	thick	green	none	none
1	E. coli	5.0	thick	tan	none	none
1	Bacillus	2.5	bloody-necrotic tissue	bloody	none	clear
1	Bacillus	1.2	thick	brown	none	clear
1	E. coli	3.7	creamy white pus surrounded with albumin-like sub.	white	none	none
1	S. aureus	3.7	thick	white	none	clear
1	Diphtheroid 130	2.5	thick	green	none	clear
1	Diphtheroid 75	2.5	thick	white	none	none
1	Diphtheroid 221	2.5	thick	green	none	none

The organisms designated as bacteroides had the following colonial and cellular morphology:

Colonial morphology--form - circular; surface - smooth; edge - entire to irregular; elevation - convex; color - gray-green to white; hemolysis - variable.

Cellular morphology--Upon initial isolation most cultures were highly pleomorphic varying in diameter from 0.5 u to 1.5 u and in length from 1.0 u to 1000 u (Figure 2). After several transfers and storage at -20 C, many of the isolates were observed as short rods with no evidence of pleomorphism (Figures 3, 4, and 5).

The taxonomy of the Gram negative anaerobes has been unresolved since a member of this group of organisms was first described by Koch in 1881. Prevot (65) and Breed et al. (9) based their generic designations on morphology but disagreed on the nomenclature and number of genera in the family Bacteroidaceae.

Breed et al. described Sphaerophorus and Bacteroides as follows:

Sphaerophorus--"Straight or curved rods, with rounded ends, which show a marked pleomorphism; filamentous and branching forms occur. Gram negative. May or may not require enriched culture media. Usually ferment glucose, rarely lactose or sucrose. Nitrites not produced in peptone media. Anaerobic. Found in the alimentary and urogenital tracts of man and other animals; also found in various gangrenous or purulent infections in man."

Bacteroides--Rods, with rounded ends, occurring singly, in pairs or in short chains; sometimes pleomorphic. Some species are encapsulated. Gram negative. May or may not require enriched culture media.

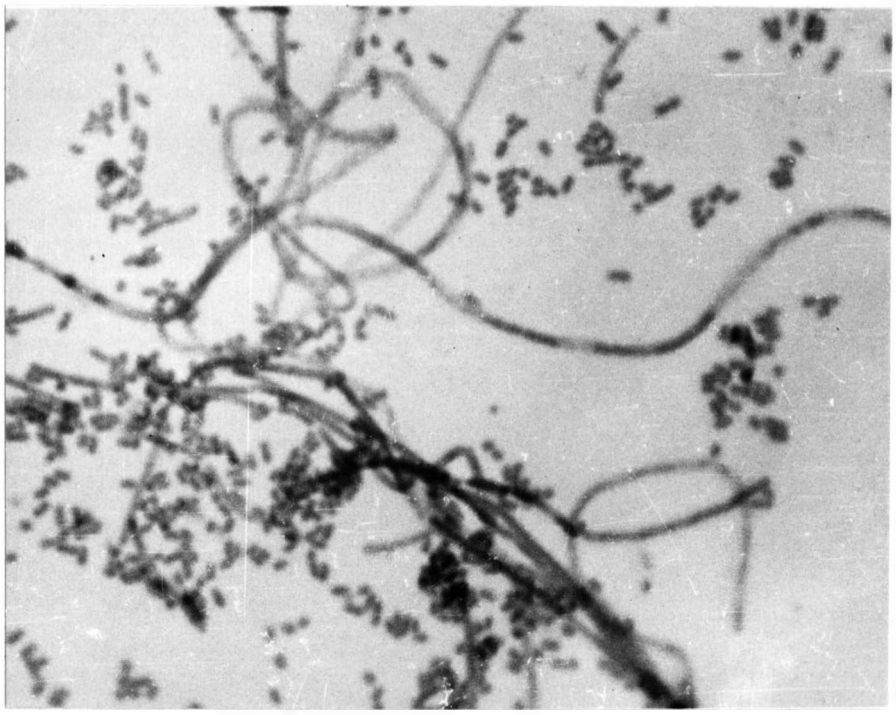

Figure 2. Initial isolate of bacteroides culture from beef liver abscess 4000X

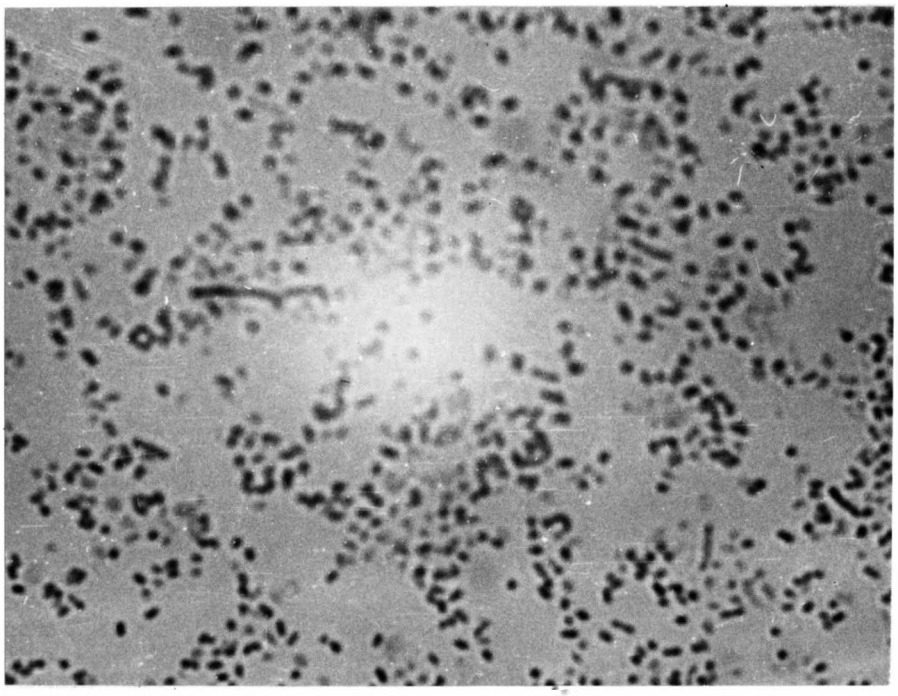

Figure 3. Bacteroides after 12 hrs anaerobic incubation in TTST at 37 C 4000X

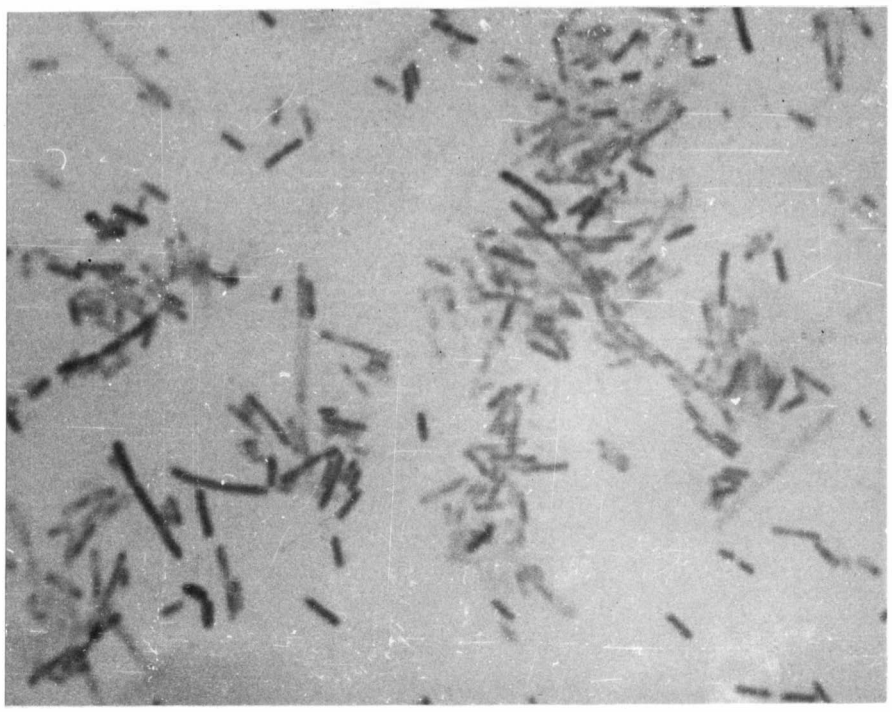

Figure 4. Bacteroides after 48 hrs anaerobic incubation in TTST at 37 C 4000X

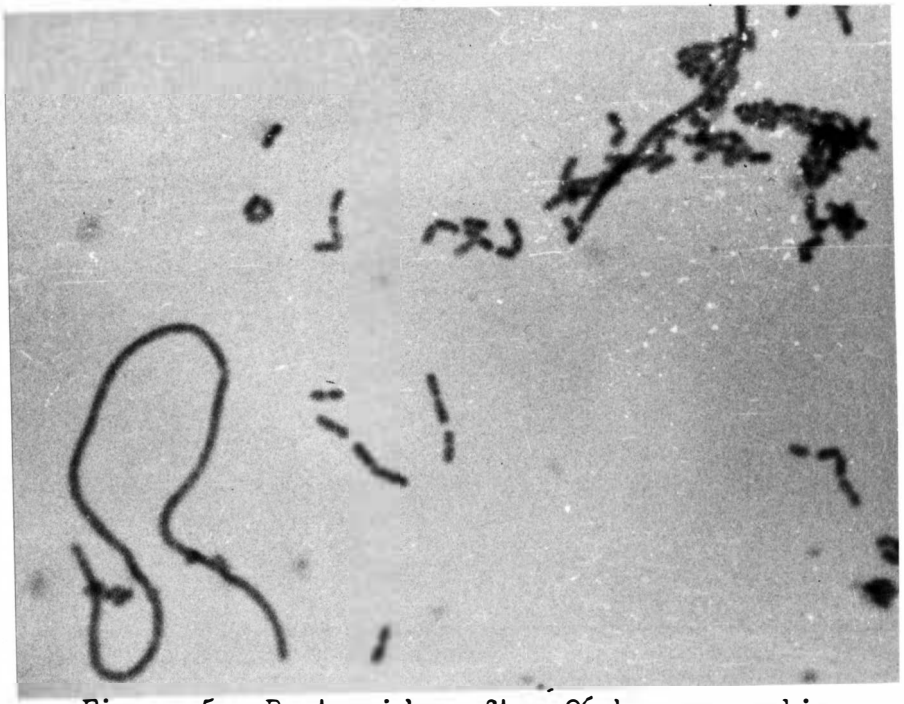

Figure 5. Bacteroides after 96 hrs anaerobic incubation in TTST at 37 C 4000X

Usually ferment glucose, rarely lactose or sucrose. Nitrites not produced from nitrates. Gas may or may not be produced in peptone media. Anaerobic. Found in the alimentary and urogenital tracts of man and other animals; some species are pathogenic."

The pleomorphic forms observed on initial isolation were more characteristic of the genus Sphaerophorus, but the short, stable forms of the same organisms observed later were more characteristic of the genus Bacteroides.

It is obvious that genus differentiation cannot be based on such an unstable characteristic as morphology or on the biochemical tests listed by Breed et al. In an effort to find a solution to this dilemma, Beerens (4) proposed the following taxonomic system for the Bacteroidaceae:

I. Threonine utilized:

A. No spindle forms
Genus I — Bacteroides
species type: B. necrophorus

B. Spindle-shaped rods
Genus II — Fusiformis
species type: F. fusiformis

II. Threonine not utilized:

A. Does not produce butyric acid starting from glucose
Genus III — Eggerthella (new genus)

a) produces aceto-propionic acid from glucose:

1. Growth favored by bile
species type: E. convexa

2. Growth not favored by bile
species type: E. sp (Barnes & Goldberg)

b) produces formic-acetic acid from glucose
species type: E. clostridiiformis

B. Produces butyric acid starting from glucose

a) Size greater than 0.3 u
Genus IV Ristella
species type: R. melanogenica

b) Size smaller than 0.15 u
Genus V Dialister
species type: D. pneumosintes.

Barnes, Impey, and Goldberg (26) differentiated Sphaerophorus necrophorus and Bacteroides convexus using the methods described by Beerens. However, these investigators used only one organism of each species in their tests so further confirmatory studies must be made with larger numbers of isolates. Goldberg (28) stated that the system as proposed by Beerens has not as yet gained acceptance. Therefore, it was his recommendation that the Gram negative anaerobes isolated in this study be designated as bacteroides. In this study bacteroides is a term used to denote any of the obligately anaerobic Gram negative bacteria which are in the family Bacteroidaceae (3).

In the literature the causative agent of beef liver abscesses is reported as S. necrophorus (27, 33, 55). The characteristics of S. necrophorus would place it in the group bacteroides. Therefore, the results of this study, which show that a large number of beef liver abscesses contain bacteroides, are consistent with the reports of earlier workers.

Diphtheroids were found in 15 abscesses and were the only organisms isolated in 6 cases. (A discussion of the diphtheroids can be found in the section on swine udders.)

Other organisms were found in 12 abscesses and were isolated in pure culture in only 6 cases.

One Streptococcus sp. and 2 Staphylococcus aureus isolations were made. Only one Micrococcus sp. was isolated (this genus is generally considered to be nonpathogenic).

Four Bacillus sp. were isolated, none of which were morphologically characteristic of B. anthracis which is the only Bacillus sp. generally considered to be pathogenic for mammals.

E. coli was found in 4 abscesses, 2 of which were in pure culture.

S. aureus and Micrococcus sp. were identified according to the techniques recommended by the Subcommittee on Taxonomy of Staphylococci and Micrococci (74). Bacillus species were identified according to Bergey's Manual of Determinative Bacteriology (9) and those organisms in the family Enterobacteriaceae were named according to the methods of Edwards and Ewing (20).

The condition of beef liver abscess was generally characterized by the presence of one to many individual abscesses from 1 to 4 inches in diameter. The abscesses were located either on the surface or deep within the liver. The abscesses were generally well encapsulated with granulation tissue which formed a dense connective tissue wall around the exudate. The exudate was generally greenish to tan in color.

It was impossible to distinguish the type of bacterial infection involved by opening the abscesses and observing gross pathology. In addition, the gross pathology of the individual abscesses was

indistinguishable regardless of the animal or organ source. The photographs in Figures 6, 7, and 8 show the type of abscess found in the majority of cases.

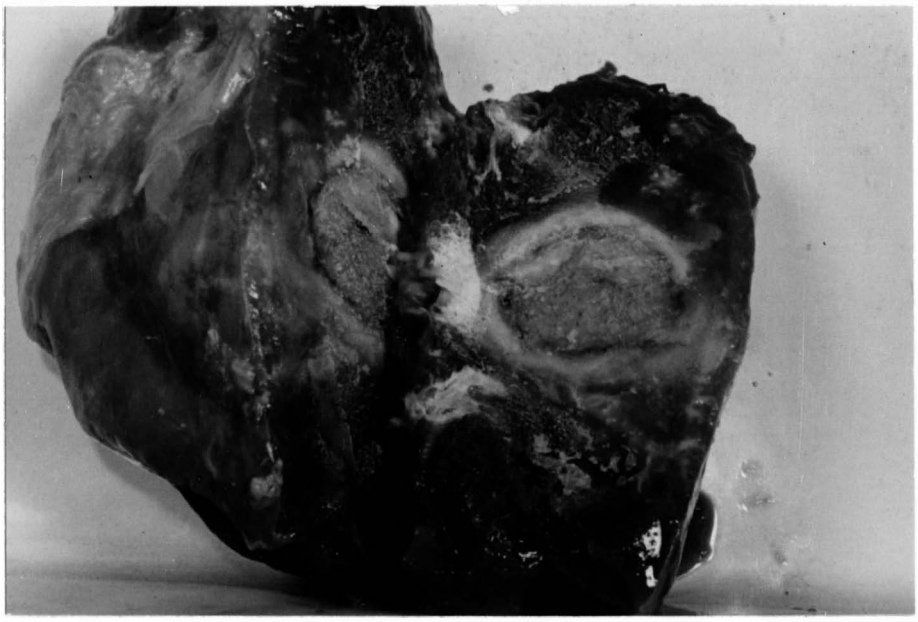

Figure 6. Beef liver abscess

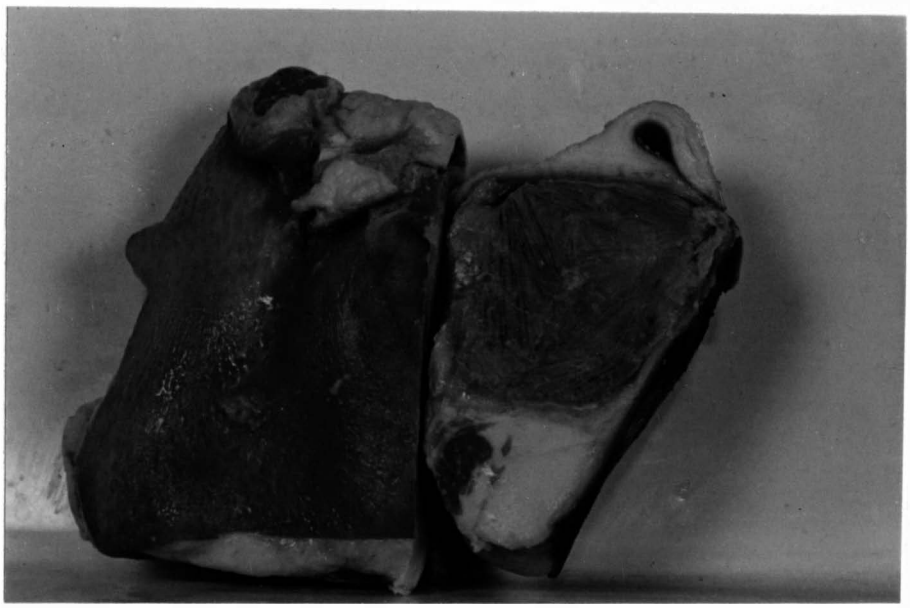

Figure 7. Swine jowl abscess

Figure 8. Swine udder abscess

Beef Jowl Abscesses

Of the 2,848 cattle observed during the collection period, only one had an infection of the jaw bone. This particular specimen was not available for study because it was mistakenly discarded by one of the workmen. Therefore, all of the 22 beef jowl abscesses studied were infections of the soft tissues (Table 4). Fifteen of the 22 abscesses contained Actinobacillus lignieresii with 14 of the 15 in pure culture. Actinobacillus lignieresii was identified according to Breed et al. (9). The organisms had the following characteristics:

potato slant--growth

litmus milk--growth with a slight reddening after 7 days

glucose broth--acid but no gas

nitrate broth--not reduced

morphology--Gram negative rods which showed bipolar staining, usually diplobacillus connected by a plasmodesma. In older cultures, involution forms occurred (Figure 9 and Figure 10).

As pointed out in the literature review, A. lignieresii was commonly reported as the etiological agent of beef jowl abscesses. These reports were substantiated in this study by the fact that A. lignieresii was isolated from 68.2% of the cases.

Streptococci were isolated in pure culture from 6 cases (27.2%) of beef jowl abscesses. Since streptococci have not been commonly reported from beef jowl abscesses, it is possible that streptococcus infections are more important than was previously believed.

Table 4. Isolations from Beef Jowl Abscesses

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis
8	*Actinobacillus*	2.5 - 10.0	thick	green	none	none
2	*Actinobacillus*	5.0 - 7.6	thick	bloody	none	none
3	*Actinobacillus*	10.0	thick	tan	none	none
1	*Actinobacillus*	2.5	thick	yellow	none	none
1	*Actinobacillus*	5.0	thick	green	none	none
1	*S. aureus*	5.0	thick	green	none	clear
1	*Micrococcus*	6.3	watery	white	none	none
2	Streptococci	7.6	thick	green	none	clear
4	Streptococci	2.5 - 10.0	thick	green	none	none

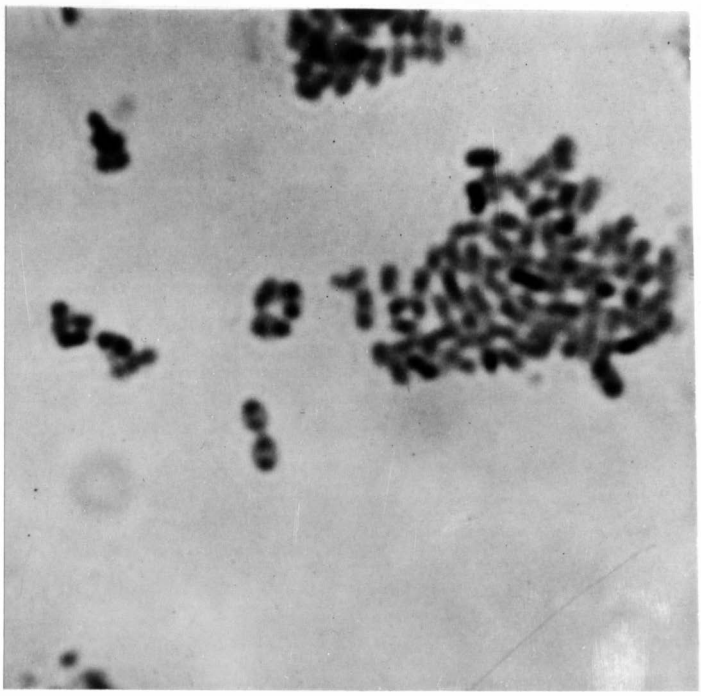

Figure 9. Gram stain of A. lignieresii showing plasmodesma and bipolar staining 4000X

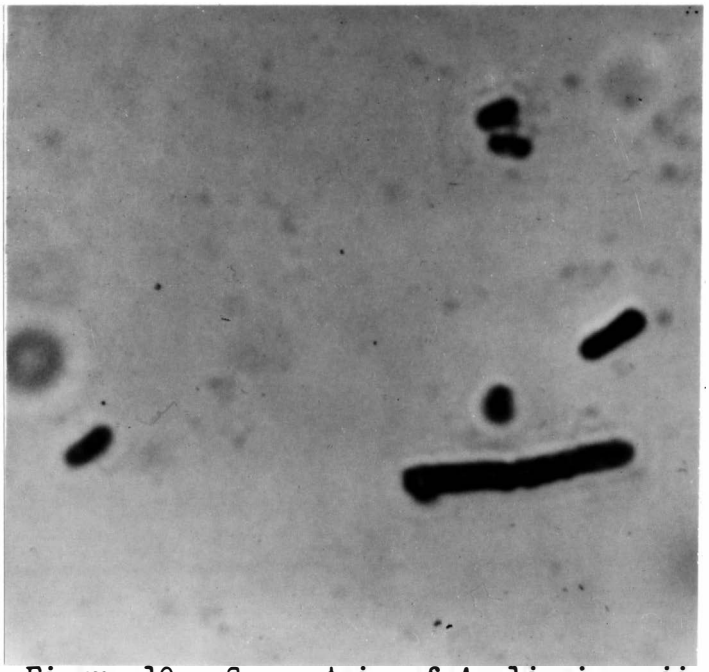

Figure 10. Gram stain of A. lignieresii involution forms 4000X

Swine Jowl Abscesses

Streptococci were isolated from swine jowl abscesses in 39 out of 55 cases in this study (Table 5). Organisms were identified as streptococci by the presence of Gram positive cocci in chains. The work of recent workers indicates that the streptococci found in swine jowl abscesses are predominately Group E Streptococcus (14, 19, 58, 77). Therefore, a sampling of 10 of the streptococci was tested against Group E antiserum by the precipitin method. Of those tested, 9 proved to be Group E Streptococcus. Therefore, it is probable that most of the streptococci isolated from swine jowl abscesses were Group E.

Collier (13) reported a combined incidence rate of 13.2% for Corynebacterium sp. and A. bovis, but diphtheroids were isolated from only 7.3% of the swine jowls in this study. Organisms from the family Enterobacteriaceae were isolated in 14.54% of the cases whereas Collier reported 9.34%. The major difference between Collier's results and the data reported in this study was in the number of obligate anaerobes isolated. Collier reported only a 0.41% Sphaerophorus necrophorus incidence compared to 14.54% bacteroides found in the samples studied here. This is due to the use of anaerobic techniques in this study compared to the aerobic techniques used by Collier.

Table 5. Isolations from Swine Jowl Abscesses

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis
31	streptococci	1.2 - 7.6	creamy to thick	greenish	none	clear
2	streptococci	7.6	creamy to thick	tan		clear
1	streptococci	7.6	watery	white	none	clear
	Diphtheroid 24	7.6	watery	white	none	none
1	streptococci	5.0	thick	black	none	none
1	streptococci	5.0	thick	green	none	clear
	streptococci	5.0	thick	green	none	none
1	streptococci	5.0	thick	green	none	clear
	streptococci	5.0	thick	green	none	none
1	streptococci	3.7	watery	red	strong	clear
	streptococci	3.7	watery	red	strong	clear
	streptococci	3.7	watery	red	strong	clear
1	streptococci	7.6	creamy	green	none	clear
	streptococci	7.6	creamy	green	none	none
1	streptococci	7.6	thick	green	none	clear
	streptococci	7.6	thick	green	none	none
	streptococci	3.7	thick	green	none	clear
	bacteroides	3.7	thick	green	none	none
1	bacteroides	3.7	watery	red	none	clear
	bacteroides	3.7	watery	red	none	none

Table 5. (continued)

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis
1	bacteroides	3.7	watery	red	none	clear
	bacteroides	3.7	watery	red	none	none
2	bacteroides	3.7	creamy	tan	none	none
1	bacteroides	7.6	thick	red	none	clear
1	bacteroides	7.6	watery	red	none	none
1	*Bacillus*	5.0	thick	green	none	none
1	*Pasteurella multocida*	10.0	thick	green	none	none
1	Diphtheroid 241	7.6	thick	green	none	clear
1	Diphtheroid 279	5.0	thick	green	none	clear
1	*Pasteurella multocida*	3.7	thick	green	none	none
1	*Proteus mirabilis*	2.5	thick	green	none	clear
1	Diphtheroid 13	7.6	mucous	green	none	none
1	*Proteus mirabilis*	5.0	creamy	green	none	none
1	*E. coli*	5.0	mucous	green	none	none

Swine Udder Abscesses

Sixty-one swine udder abscesses were cultured (Table 6). Diphtheroids were isolated in pure culture from 16 abscesses and from mixed infections in 15 abscesses; bacteroides were found in pure culture in 9 abscesses and 11 in mixed infections; streptococci were isolated in pure culture from 6 abscesses and from 7 mixed infections; Enterobacteriaceae were isolated 11 times, all in mixed infections; S. aureus was isolated from one abscess; and a Bacillus sp. was isolated from 1 abscess.

The Diphtheroids--In this study the term diphtheroids includes all of the organisms morphologically similar to the genus Actinomyces. The diphtheroids were classified using the methods of the "International Subcommittee for the Identification of the Actinomycetes, Subgroup on Microaerophilic Actinomyces" (75), the National Communicable Disease Center's Manual for Course No. 837 (47), and the techniques recommended by George et al. (25). The following classification system, as recommended by Pine and Georg (64), was employed for genus designation:

Kingdom - Plant

Division - Protophyta (Sachs, 1874, Emend. Drassilnikov, 1949)

Class - Schizomycetes (Von Naegeli, 1857)

Order - Actinomycetales (Buchanan, 1917)

Order Actinomycetales

> Organisms composed of elongated cells that have a definite tendency to branch. Hyphae do not exceed 1.5 μ in width and are mostly 1.0 u or less in diameter.

Table 6. Isolations from Swine Udder Abscesses

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis*
1	Diphtheroid 25	5.0	creamy	green	none	clear
1	Diphtheroid 31	3.0	thick	green	none	clear
1	Diphtheroid 50	11.4	mucoid	green	none	clear
1	Diphtheroid 52	5.0	mucoid	green	none	none
1	Diphtheroid 49	2.5	thick	green	none	clear
1	Diphtheroid 167	5.0	thin	tan	none	clear
1	Diphtheroid 168	5.0	thin	tan	none	none
1	Diphtheroid 206	5.0	thick	green	none	clear
1	Diphtheroid 270	2.5	thick	tan	none	clear
1	Diphtheroid 213	5.0	thick	tan	none	clear
1	Diphtheroid 51	15.0	thick	green	none	none
1	Diphtheroid 106	5.0	thick	tan	none	clear
	Diphtheroid 107	5.0	thick	tan	none	none
1	Diphtheroid 303	10.0	creamy	tan	none	clear
	Diphtheroid 304	10.0	creamy	tan	none	none
1	Diphtheroid 325	3.7	thin-watery	tan	stunk	clear
	Diphtheroid 326	3.7	thin-watery	tan	stunk	none

Table 6. (continued)

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis*
1	Diphtheroid 332	10.0	creamy	tan	none	clear
	Diphtheroid 333	10.0	creamy	tan	none	none
1	Diphtheroid 43	3.7	thick	green	none	clear
	bacteroides	3.7	thick	green	none	none
1	Diphtheroid 329	5.0	creamy	tan	putrid	clear
	bacteroides	5.0	creamy	tan	putrid	none
1	Diphtheroid 230	3.7	thick	green	none	none
	bacteroides	3.7	thick	green	none	none
1	Diphtheroid 94	7.6	thick	tan	none	clear
	bacteroides	7.6	necrotic tissue (tct)	tan	none	none
1	Diphtheroid 97	10.0	tct	tan	putrid	clear
	bacteroides	10.0	tct	tan	putrid	none
1	Diphtheroid 103	15.0	tct	tan	none	none
	bacteroides	15.0	tct	tan	none	clear
1	Diphtheroid 42	10.0	nucous	green	none	clear
	Micrococcus	10.0	mucous	green	none	none
1	Diphtheroid 271	5.0	thick	tan	none	clear
	Aerobacter aerogenes	5.0	thick	tan	none	none
1	Diphtheroid 236	2.5	thick	tan	none	clear
	S. aureus	2.5	thick	tan	none	clear

Table 6. (continued)

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis*
1	Diphtheroid 327	3.7	watery	tan	none	clear
1	Diphtheroid 209	2.5	thick	tan	none	clear
	E. coli	2.5	thick	tan	none	none
1	Diphtheroid 232	1.2	thick	tan	none	clear
	Micrococcus	1.2	thick	tan	none	none
1	Diphtheroid 234	1.2	thick	tan	none	none
	Bacillus	1.2	thick	tan	none	none
1	Diphtheroid 108	7.6	thick	tan	none	clear
	bacteroides	7.6	thick	tan	none	clear
1	*Aerobacter aerogenes*	5.0	thick	green	none	clear
	Micrococcus	5.0	thick	green	none	none
1	Diphtheroid 166	5.0	thick	tan	putrid	none
	Diphtheroid 165	5.0	thick	tan	putrid	none
4	bacteroides	3.7	watery	white	none	none
3	bacteroides	7.6	thick	tan	none	none
2	bacteroides	5.0	thick	tan	none	clear
1	bacteroides	5.0	thick	green	slight	none
2	*S. aureus*	2.5	thick	tan	none	clear

Table 6. (continued)

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis*
	Diphtheroid 322	7.6	thick	green	none	none
1	Streptococci	7.6	thick	green	none	clear
	Bacillus	7.6	thick	green	none	none
	streptococci	5.0	necrotic - pus	green	none	none
1	Bacillus	5.0	nec. no pus	green	none	none
	aerobacter aerogenes	7.6	thick	tan	none	none
1	E. coli	7.6	thick	tan	none	none
	aerobacter aerogenes	5.0	thin	tan	none	none
1	proteus mirabilis	5.0	thin	tan	none	none
	streptococci	2.5	cheesy	green	none	none
1	E. coli	2.5	cheesy	green	none	none
1	bacteroides	5.0	thick	green	none	none
	streptococci	5.0	thick	green	none	none
	bacteroides	7.6	thick	tan	none	none
1	E. coli	7.6	thick	tan	none	none
	bacteroides	10.0	thick	tan	putrid	clear
1	streptococci	10.0	thick	tan	putrid	none
	streptococci	7.6	watery	tan	none	none
1	streptococci	7.6	watery	tan	none	none

Table 6. (continued)

No. of abscesses	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis*
1	bacteroides	2.5	thick	green	none	none
	streptococci	2.5	thick	green	none	clear
1	Aerobacter aerogenes	2.5	creamy	tan	none	none
1	E. coli	5.0	watery	bloody	none	none
1	streptococci	3.7	thick	tan	none	none
1	streptococci	5.0	thick	red	none	none
1	streptococci	7.6	watery	red	none	none
1	streptococci	5.0	thick	green	putrid	none
1	streptococci	12.7	thick	tan	none	clear
1	streptococci	2.5	thick	tan	none	none

*Determined by subculture on blood agar.

I. True mycelium is formed on the surface of agar media or under certain conditions in tissues. In culture, branched filaments or budding cells are always formed; serial mycelium and spores are never formed; anaerobic, facultative, and aerobic; colonies of McClung's (1954) group I. Fermentation of glucose occurs under anaerobic, microaerophilic or aerobic conditions with the production of volatile and nonvolatile acids.
Family: Actinomycetaceae

A. Anaerobic to facultative, catalase negative, true mycelium formed but may be transitory. Glucose fermented to produce characteristic amounts of volatile (formic, acetic, or propionic) acids and lactic and succinic acids.
Genus: Actinomyces

B. Facultative, catalase positive. True but transitory mycelium formed. Formic, acetic, lactic and succinic acid formed from glucose.
Genus: "Hamster organism" (Howell, 1964)
Odontomyces

C. Strict aerobe, catalase positive, true mycelium formed. Acetic and lactic acids are major products formed from glucose under aerobic conditions.
Genus: "Nocardia" dentocariosus (Roth and Thurn, 1962)

II. True primary mycelium is always formed adherent to the agar and a secondary aerial mycelium more or less perpendicular to the surface of the mycelium may or may not be formed, but "sporangia" are never formed. Cellular division by transverse wall formation only. Spores nonmotile. Strict aerobes. McClung's (1954) colony groups II and III.
Family: Streptomycetaceae

III. Mycelium rudimentary or absent. Branched cells may or may not be formed. True mycelium not formed on the surface of agar media but found occasionally in clinical materials. McClung's group I. Strict aerobes. Spores are never formed.
Family: Mycobacteriaceae

A. Cells usually acid-fast. Rod-shaped cells that do not branch under ordinary cultural conditions.
Genus: Mycobacterium

B. Cells nonacid fast. Cells generally spherical, occurring singly or in clumps
Genus: Micrococcus

IV. True primary mycelium formed with subsequent fragmentation into coccoidal cellular units. Cellular division by transverse and longitudinal cell wall formation. Secondary serial mycelium not formed. Motile spores formed by longitudinal and transverse division of hyphal structures not sporangia.
Family: Dermatophilaceae

V. A true mycelium formed; spores formed in sporangia.
Family: Actinoplanaceae

Species differentiation was accomplished by comparative studies of the isolated organism with known species.

Serology of the Diphtheroids--Precipitation reactions using the Ouchterlony technique revealed no antigenic relationship between the 11 known diphtheroid species and the 8 selected unknown isolates (Table 7). The only cross-reactions occurring within the 11 known cultures were: the antigen of A. eriksonii and the antiserum of A. bovis; the antigen of Odontomyces viscosus with the antisera of A. bovis and A. discofoliatus; and the antigen of A. bovis and A. parabifidus with the antisera of O. viscosus.

The 8 unknown isolates, against which antisera were prepared, were divided into 2 major serological groups based on reciprocal cross-precipitation reactions (Table 7). Group I consists of unknown diphtheroid 230 which is not antigenically related to any of the other isolates. Group II contains unknown diphtheroids 25, 43, 94, 108, 126, 271, and 326; these were divided into two subgroups. The organisms of Group IIa (43, 94, 108, and 271) are closely related serologically. Group IIb includes 25, 126, and 326 which appear to be of much lower antigenicity than the organisms of Group IIa. In fact, antiserum from unknown diphtheroid 326 did not react with any of the antigens, including its homologous antigen. Groups IIa and IIb possess antigenically

Table 7. Antigenic Cross Relationships of Known Diphtheroid Species and Selected Unknown Isolates*

Rabbit antisera	Soluble antigens																			
	A. bovis	A. naeslundii	A. israelii	A. eriksonii	A. odontolyticus	A. parabifidus	A. propionicus	A. discofoliatus	N. dentocariosus	O. viscosus	R. pleuriticum	25 Group IIb	43 Group IIa	94 Group IIa	108 Group IIa	126 Group IIb	230 Group I	271 Group IIa	326 Group IIb
A. bovis	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A. naeslundii	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. israelii	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. eriksonii	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. odontolyticus	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. parabifidus	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
A. propionicus	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
A. discofoliatus	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
N. dentocariosus	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
O. viscosus	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
R. pleuriticum	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
25 Group IIb	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-
43 Group IIa	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
94 Group IIa	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-
108 Group IIa	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
126 Group IIb	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-
230 Group I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
271 Group IIa	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-
326 Group IIb	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-

*Determined from precipitin patterns in agar-gel; + = precipitin reaction; 0 = no reactions.

similar components which were demonstrated when the antigens of Group IIb were allowed to react with the antisera of Group IIa organisms.

The soluble antigens of the 65 diphtheroids isolated were tested against the antisera of the 11 known species and the 8 selected unknown isolates. On the basis of the precipitin reaction, it was possible to divide the 65 unknown diphtheroids into 5 groups.

Group I (Table 8) contains 6 isolates, in addition to diphtheroid 230, which are serologically distinct from all other cultures.

Thirty-five diphtheroids were placed in Group II (Table 8) in addition to the 7 organisms previously assigned to this group. Twenty-nine of these organisms were placed in subgroup IIa. Subgroup IIb was further subdivided into groups IIb1, IIb2, and IIb3.

Subgroup IIb1 contains 6 diphtheroids which have antigens in common with 4 of the members of subgroup IIa; but, in each case, a reaction was lacking with one of the members of subgroup IIa. Subgroup IIb2 contains 2 isolates which showed precipitin lines with the antiserum prepared against 108 (subgroup IIa), but not with any of the other antisera.

Subgroup IIb3 contains 4 organisms whose antigens reacted with the antiserum prepared against 326. This is peculiar because 326 antigen did not react with its homologous antiserum. Possible explanations of this phenomenon are:

1) Isolate 326 did not release a soluble antigen into the culture medium.

Table 8. Antigenic Relationships of Unknown Diphtheroids with Antisera Prepared Against Known Species and Selected Unknowns*

Rabbit antisera	Soluble antigens: Group I							Group IIa																						Group IIb1						Group IIb2	
	230	327	333	179	130	24	144	49	51	52	103	106	111	167	206	209	233	287	289	304	325	13	18	178	243	75	31	258	259	270	273	322	241	218	255	175	253
A. bovis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. naeslundii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. israelii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. eriksonii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. odontolyticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. parabifidus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. propionicus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. discofoliatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N. dentocariosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O. viscosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R. pleuriticum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
94	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-
108	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
126	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-
230	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
273	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-
326	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 8. (continued)

Rabbit antisera	Soluble antigens																					
	Group IIb3					Group III			Group IV			Group V										
	42	79	252	260	236	221	288	168	217	279	303	107	166	97	329	142	80	122	194	234	117
A. bovis	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
A. naeslundii	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
A. israelii	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
A. eriksonii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. odontolyticus	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
A. parabifidus	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
A. propionicus	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A. discofoliatus	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
N. dentocariosus	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
O. viscosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R. pleuriticum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
94	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
108	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
126	+	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
230	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
273	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
326	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-

*Determined from precipitin patterns in agar-gel; + = precipitin reaction; 0 = no reaction.

2) An insufficient quantity of antigen was available to give a visible precipitin reaction even though antibody had been produced by the rabbit.

Group III contains 3 organisms which reacted only with the antiserum produced against A. odontolyticus.

Three organisms were placed in Group IV. These did not fall into any of the previous categories, but reacted with some antisera in all of the previous groups and with the antisera of several of the known diphtheroids.

Group V includes 9 organisms whose antigens did not react with any of the known or unknown antisera.

Confirmation of the above groupings must await further and more complete antigenic analyses of these isolates. Soluble cell extracts, from representative isolates in each group, should be used to prepare antisera for reciprocal Ouchterlony tests (61). Specificity of reactions should be determined by appropriate adsorption procedures.

Morphology of the Diphtheroids--Upon initial isolation the unknown isolates did not demonstrate the expected Gram positive branching structures. Instead, they were pleomorphic and stained weakly Gram positive to Gram negative.

After several transfers and storage at -20 C, the cultures became filamentous (Figures 11, 12) which is characteristic of the Actinomyces sp.

Comparison of cells in 24-hour microcolonies of known Actinomyces sp., Actinomyces-like organisms, and the unknown diphtheroids revealed a great deal of morphological variation. Ramibacterium pleuriticum and

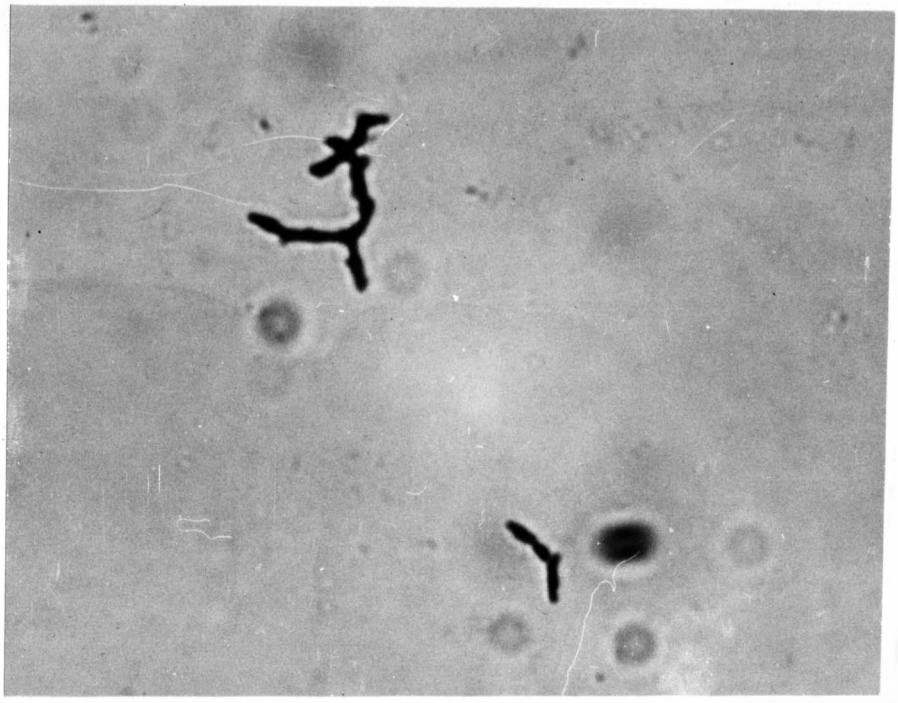

Figure 11. Gram stains of unknown cultures (327) 4000X

Figure 12. Gram stains of unknown cultures (47) 4000X

Nocardia dentocariosus (Figures 13, 14) demonstrated bacteria-like characteristics only. A. bovis, A. israelii, A. odontolyticus, and A. parabifidus were also bacteria-like but showed a morphology distinct from R. pleuriticum and N. dentocariosus (Figures 15, 16, 17, 18). Both smooth bacterial and filamentous forms were demonstrated by A. eriksonii, A. naeslundii, A. propionicus, and A. discofoliatus (Figures 19, 20, 21, 22, 23, 24, 25, 26). Spherical cells (Figure 19) were observed in several of the Actinomyces sp. Odontomyces viscosus (Figure 27) was the only organism consistently observed as a filamentous form.

The organisms in Figures 28, 29, 30, and 31 show structures which were characteristic of the organisms in Groups I and II. The smooth microcolonial cell type characteristic of A. bovis and the filamentous microcolonial cell type of A. naeslundii were demonstrated. The spherical cells, which were demonstrated in A. eriksonii, were also observed in unknown 18 (Figure 28). The frequent observation of these structures raises a question concerning their function and taxonomic significance. The swollen peripheral cells of the colony seen in diphtheroid 167 (Figure 29) were observed frequently. It is possible that these structures are related to the spherical cells; however, the spherical cells have a definite circular structure and were seldom found in association with the swollen cells.

The organisms of serological Group III (Figures 32, 33) appeared to have a microcolonial cellular morphology which was distinct from the other unknown groups. These cultures consistently demonstrated filamentous colonies after 24 hours incubation. The microcolonial cell morphology of Groups I, IV, and V was indistinguishable from that of Group II.

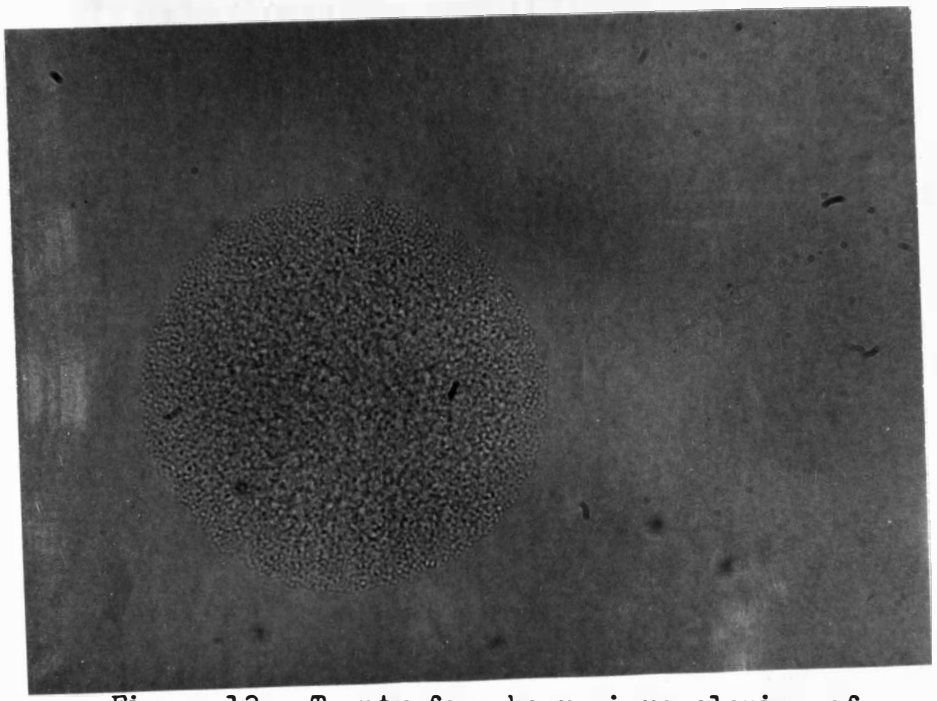

Figure 13. Twenty-four-hour microcolonies of Ramibacterium pleuriticum 2000X

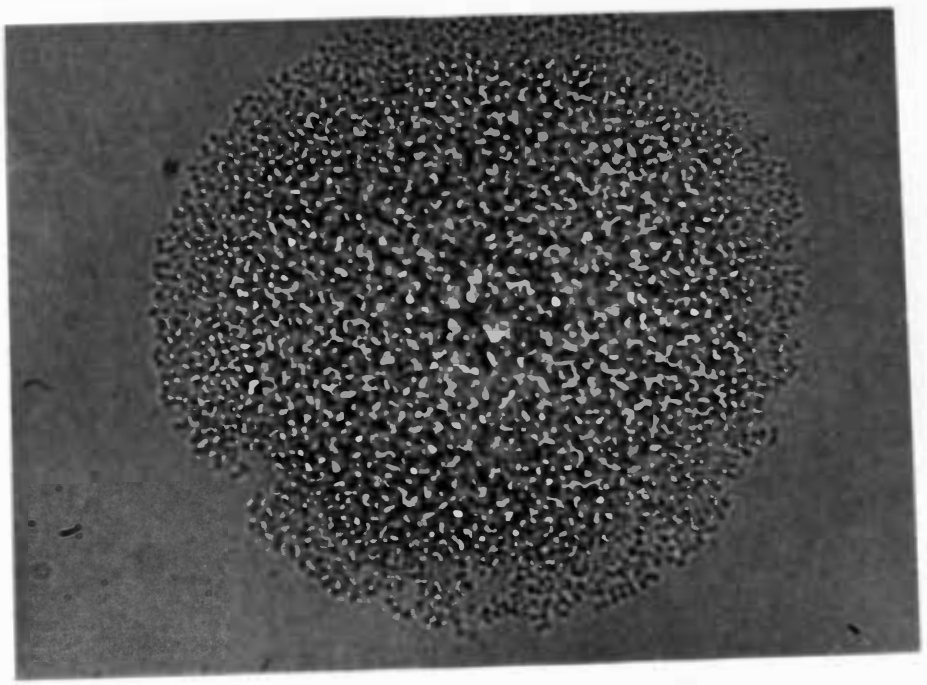

Figure 14. Twenty-four-hour microcolonies of Nocardia dentocariosis 2000X

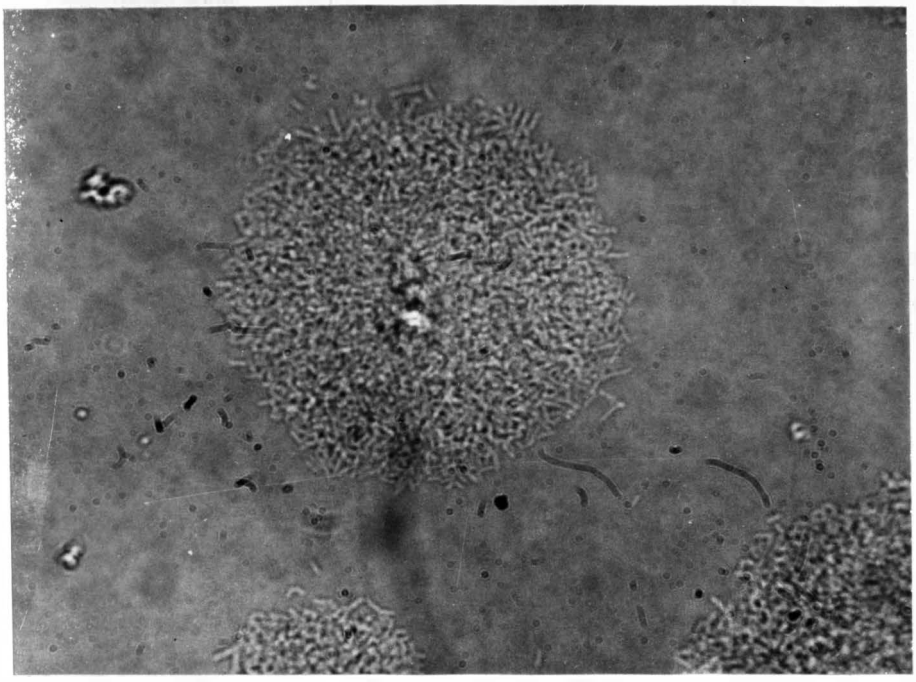

Figure 15. Twenty-four-hour microcolonies of Actinomyces bovis 2000X

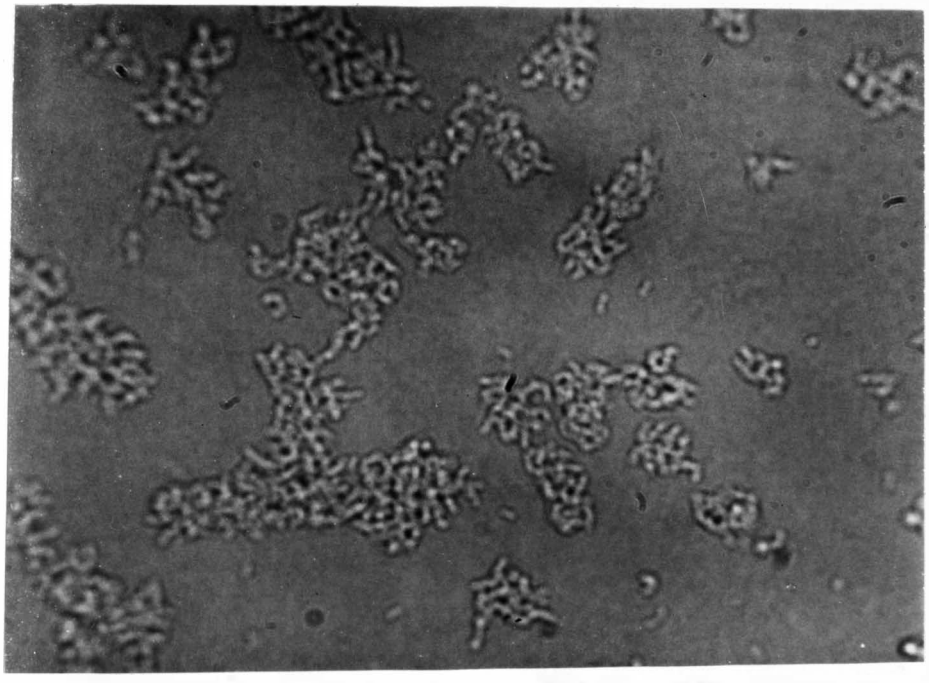

Figure 16. Twenty-four-hour micrcolonies of Actinomyces israelii 2000X

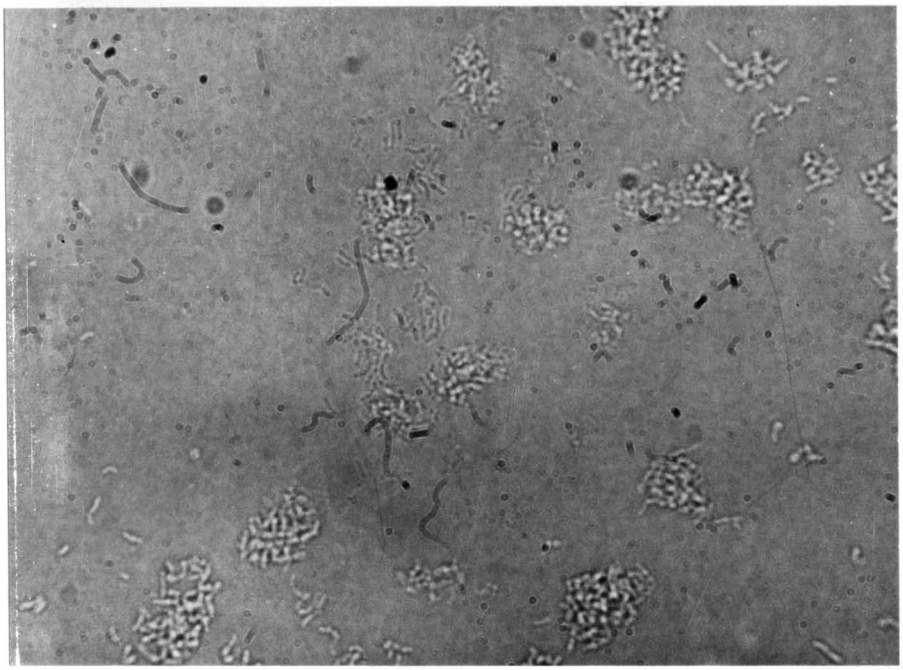

Figure 17. Twenty-four-hour microcolonies of Actinomyces parabifidus 2000X

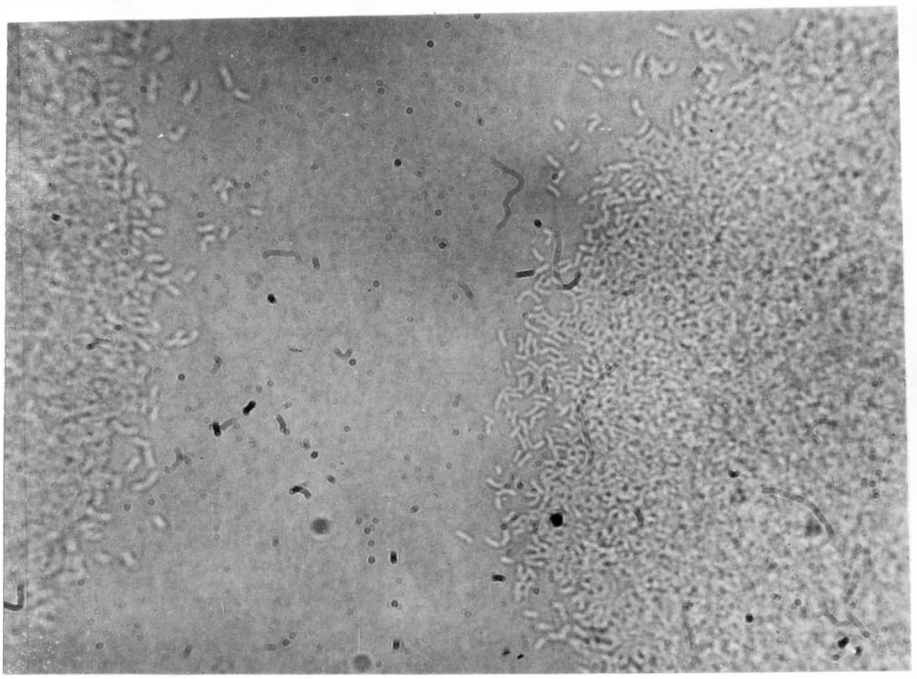

Figure 18. Twenty-four-hour microcolonies of Actinomyces odontolyticus 2000X

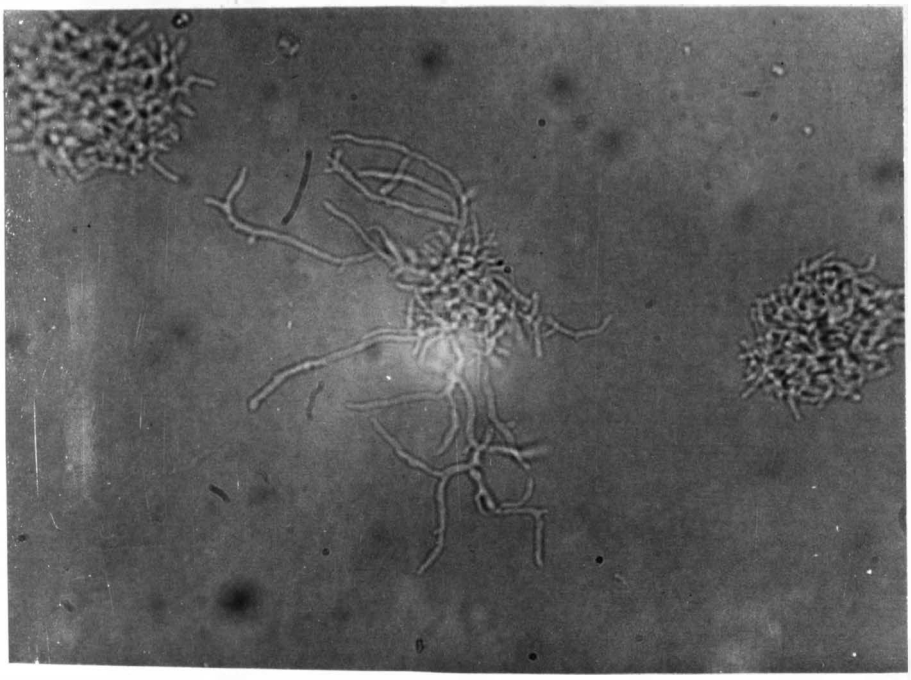

Figure 19. Twenty-four-hour microcolonies of Actinomyces naeslundii which demonstrate fungal morphology 2000X

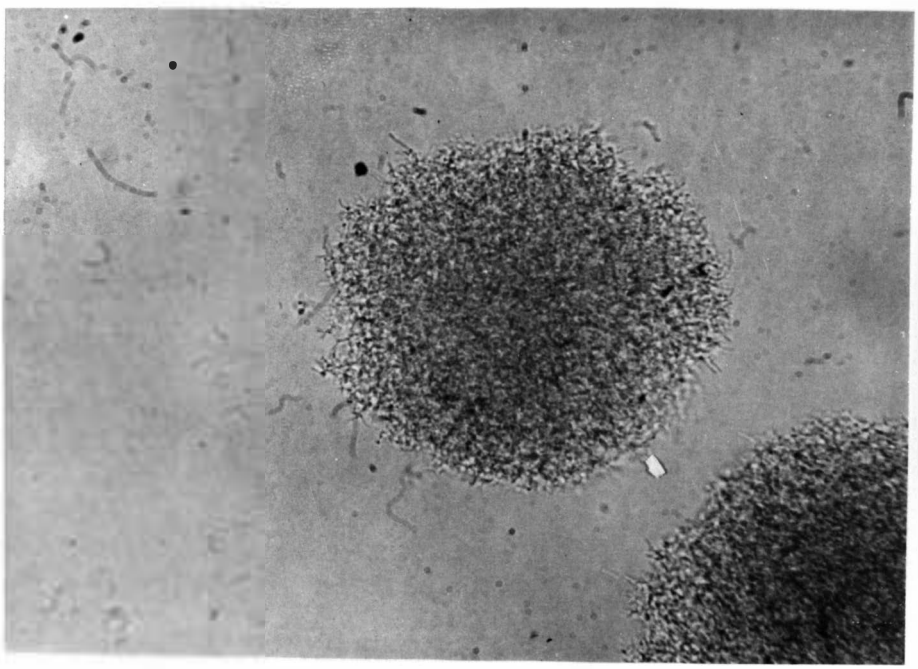

Figure 20. Twenty-four-hour microcolonies of Actinomyces naeslundii which demonstrate bacterial morphology 2000X

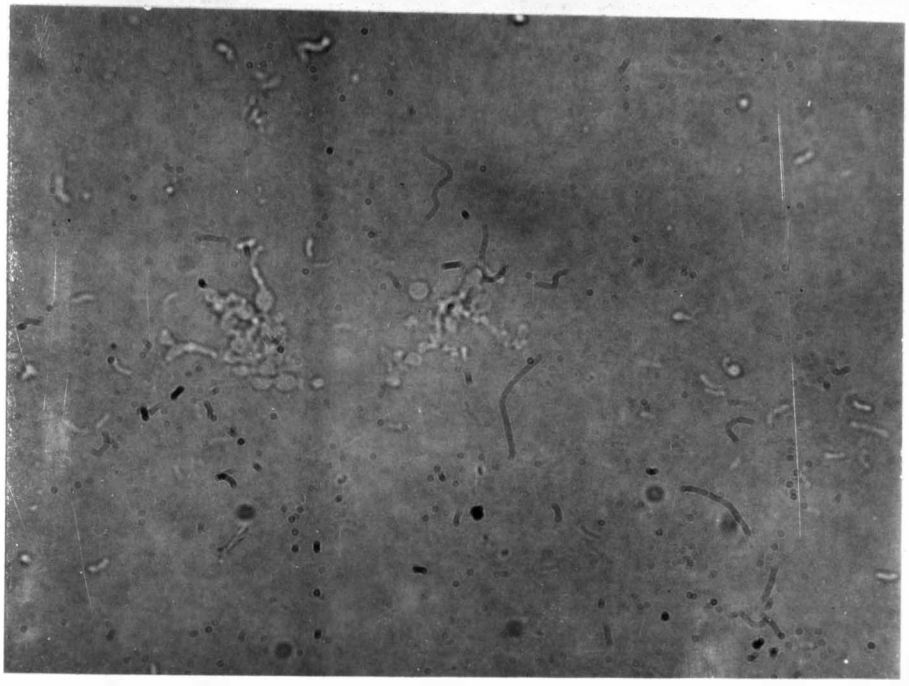

Figure 21. Twenty-four-hour microcolonies of Actinomyces eriksonii which demonstrates spherical bodies 2000X

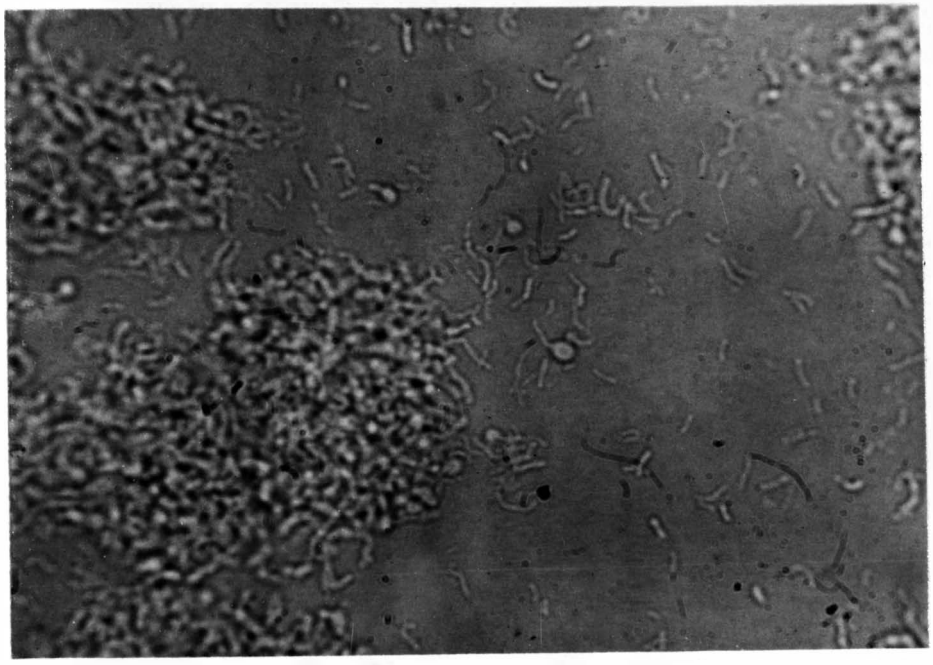

Figure 22. Twenty-four-hour microcolonies of Actinomyces eriksonii which demonstrates fungal and bacterial morphology 2000X

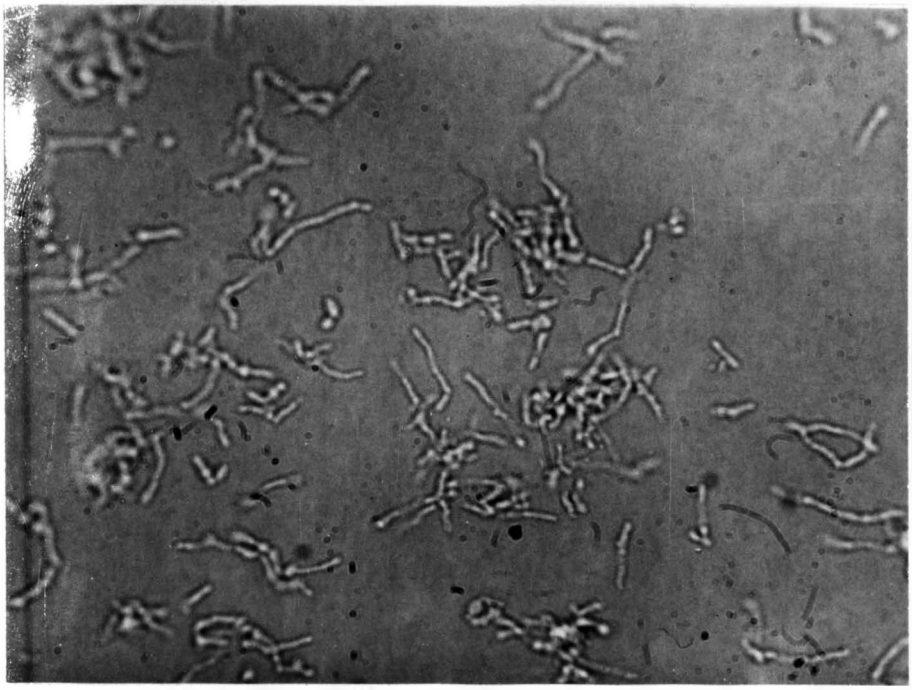

Figure 23. Twenty-four-hour microcolonies of Actinomyces propionicus which demonstrates fungal morphology 2000X

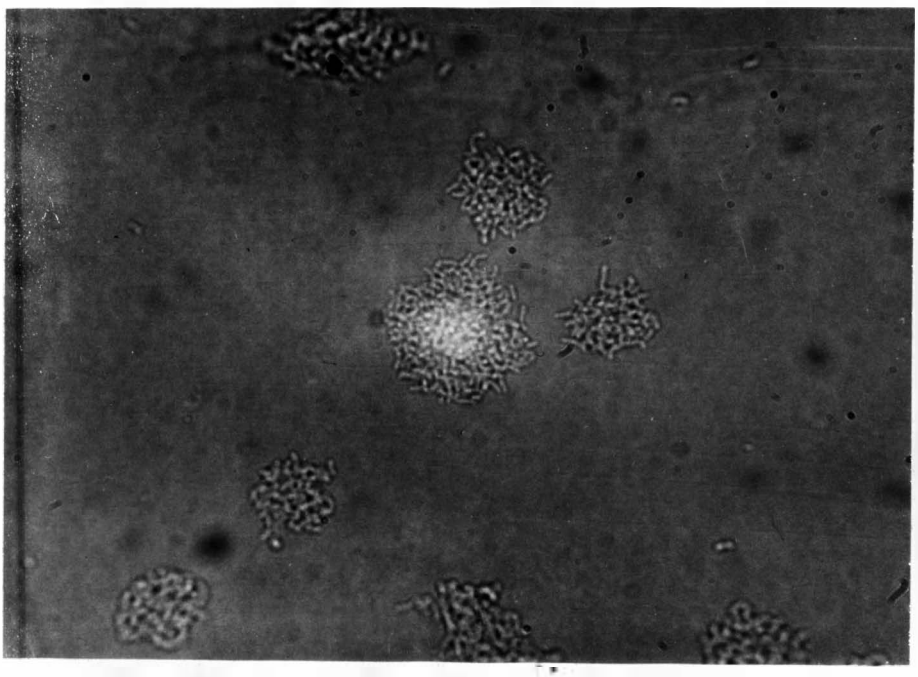

Figure 24. Twenty-four-hour microcolonies of Actinomyces propionicus which demonstrates bacterial morphology 2000X

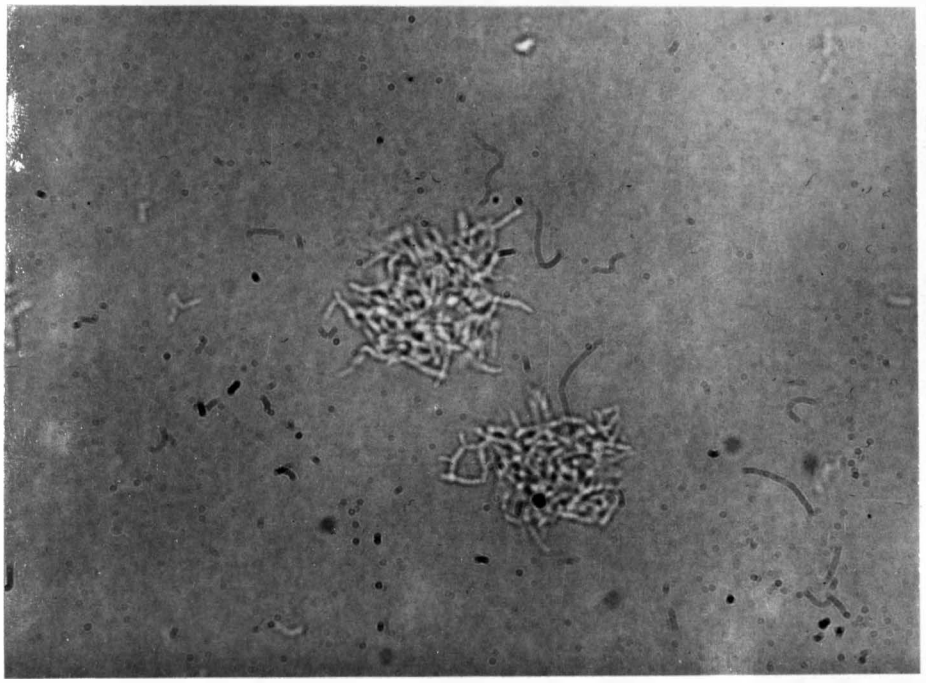

Figure 25. Twenty-four-hour microcolonies of Actinomyces discofoliotus which demonstrates fungal morphology 2000X

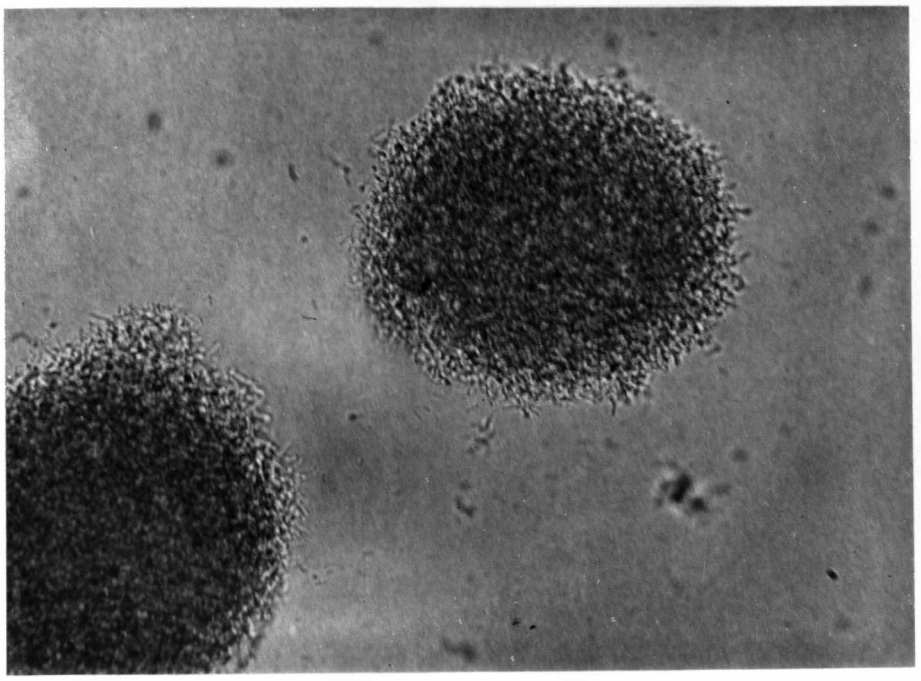

Figure 26. Twenty-four-hour microcolonies of Actinomyces discofoliotus which demonstrates bacterial morphology 2000X

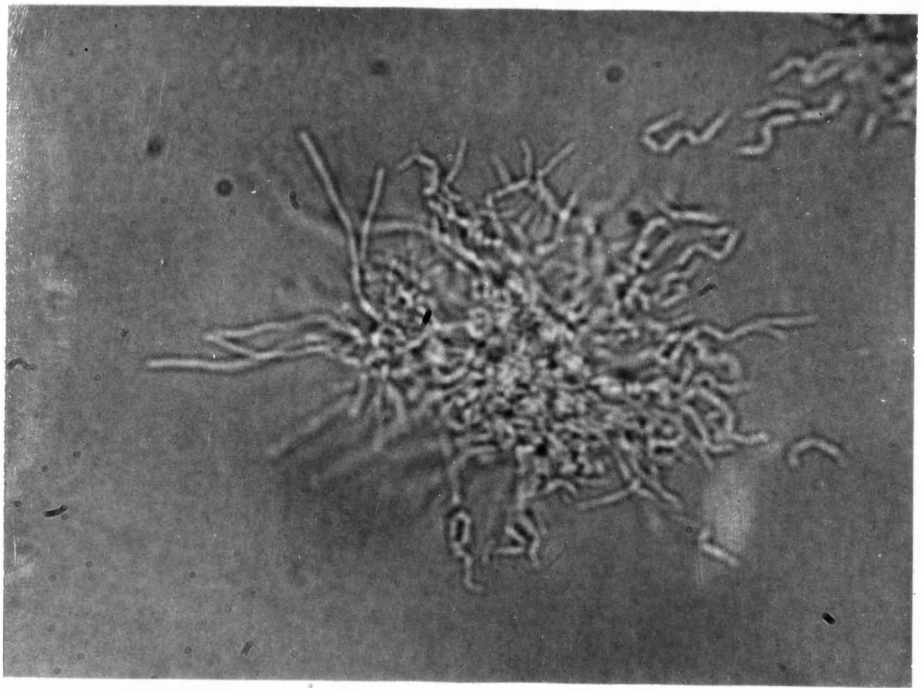

Figure 27. Twenty-four-hour microcolonies of Odontomyces viscosus which demonstrates fungal form 2000X

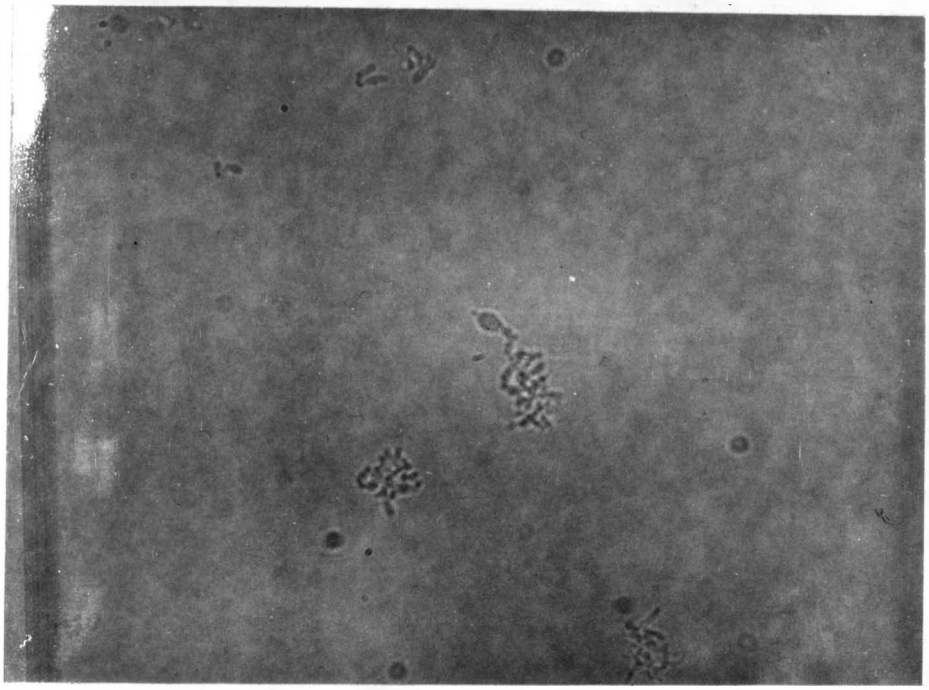

Figure 28. Microcolony morphology of unknown 18 2000X

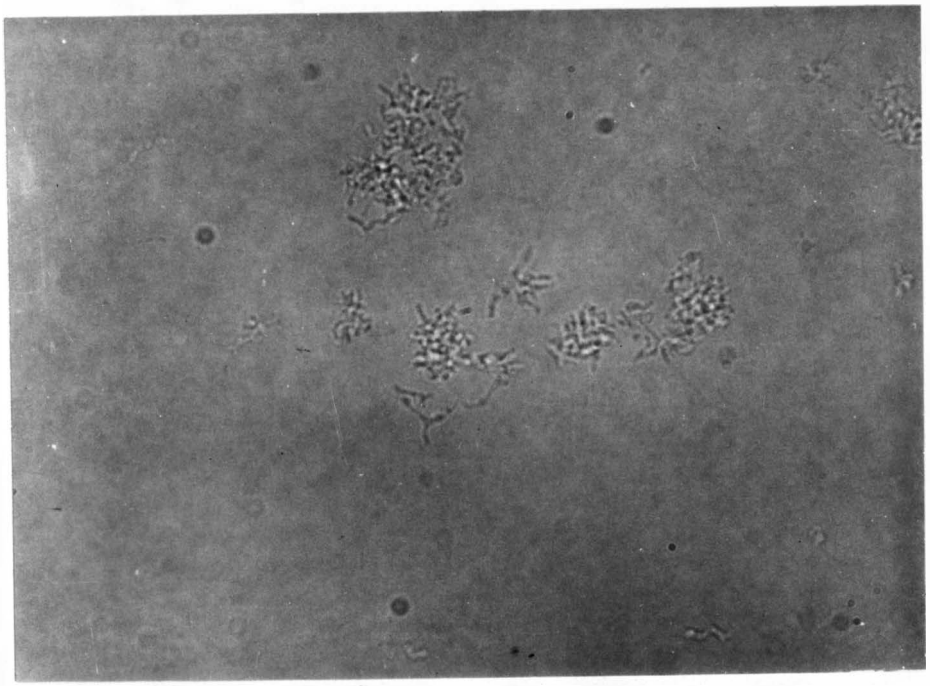

Figure 29. Microcolony morphology of unknown 75 2000X

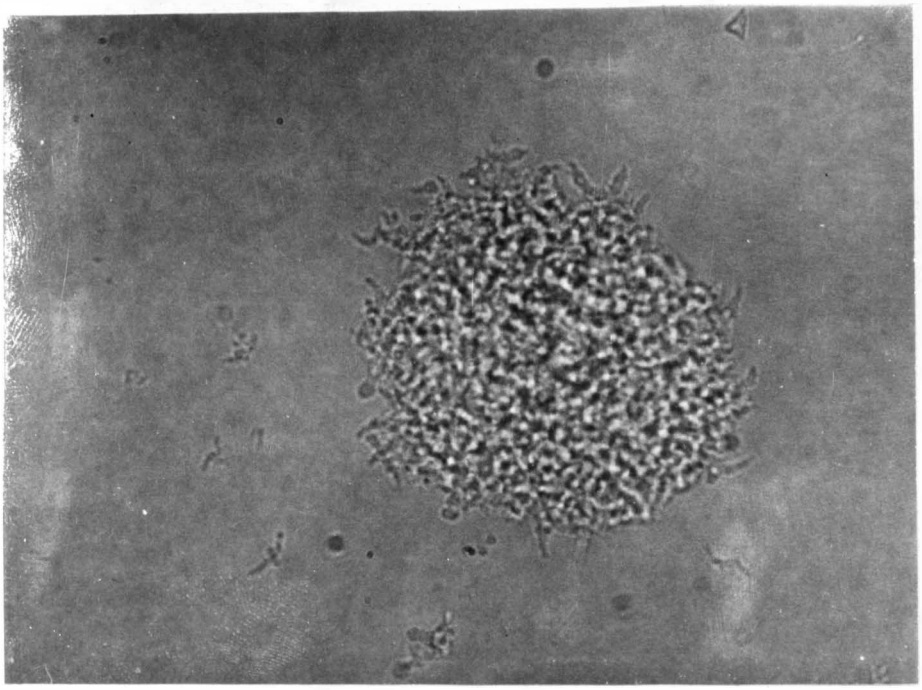

Figure 30. Microcolony morphology of unknown 167 2000X

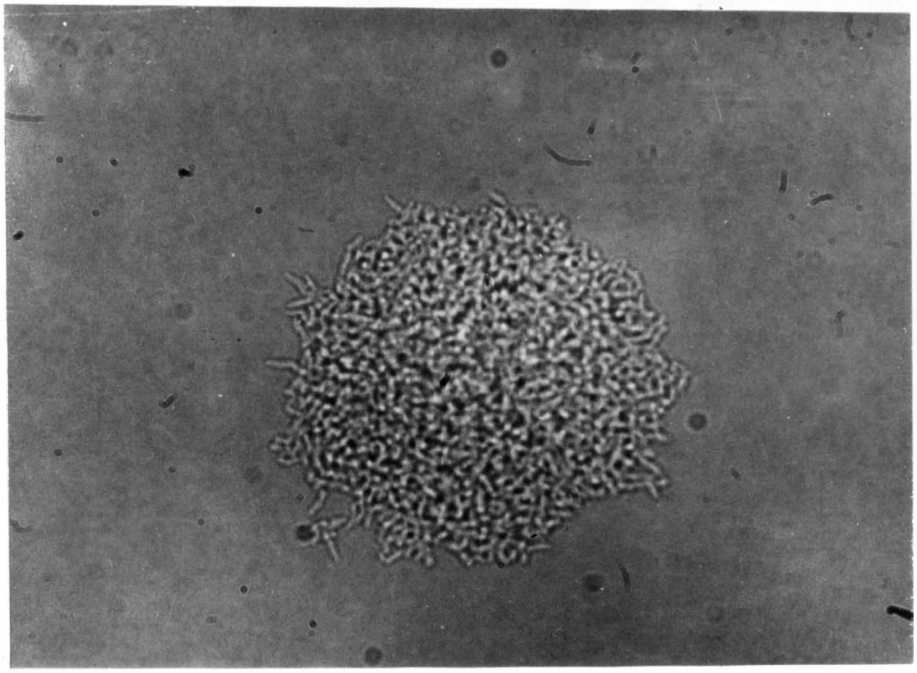

Figure 31. Microcolony morphology of unknown 206 2000X

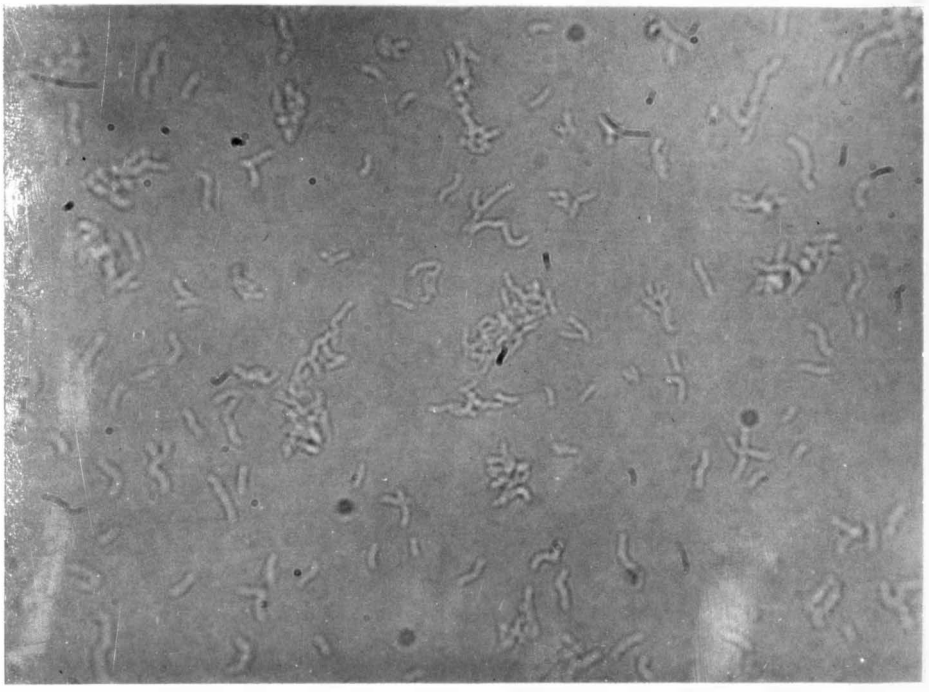

Figure 32. Microcolony morphology of unknown 221 2000X

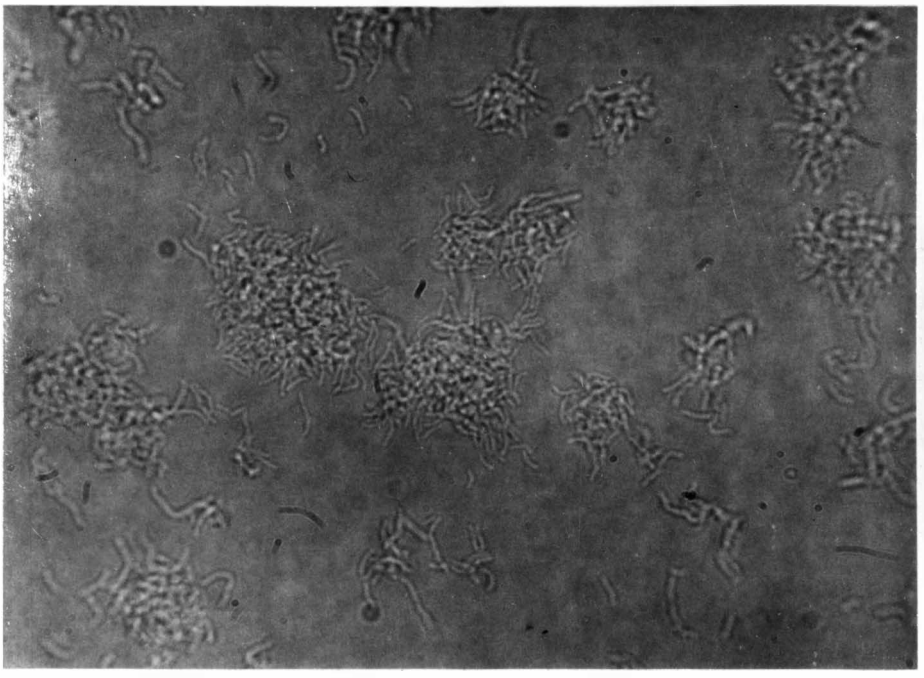

Figure 33. Microcolony morphology of unknown 221 2000x

The variation in morphology from bacterial to filamentous form presents interesting questions in relationship to biochemical activities. Grasser (29) demonstrated, in his work with A. suis and A. israelii, that the variation in morphology did not result in a variation in antigenic structure as demonstrated with the agglutination tests. However, this morphological change was accompanied by a change in the ability of A. israelii to ferment mannitol, glycerol, and inulin and in the ability of A. suis to ferment adonitol, arabinose, rhamnose, saccharose, and sorbitol. Variations in cell morphology may explain the inconsistencies in fermentation reactions shown in this study and in the findings of other investigators.

Pengrum (62) converted A. israelii from the rough form to the smooth form by inoculation on the chorioalantoic membrane of the chick embryo. On first isolation from the infected eggs, the obligate anaerobe, A. israelii, grew on blood agar aerobically as well as anaerobically. After subculture in anaerobic broth, it reverted to an anaerobic rough form. The actual mechanism of the change from rough to smooth form is not known. It is possible that the change is related to cell wall synthesis. If this is the case, a variation in the cell's ability to ferment a sugar could be related to the change in morphology by effecting a change in the materials available for cell wall synthesis, or the change in cell wall structures could effect a change in permeability.

The Actinomycetales are considered to be phylogenetically located between the bacteria and the fungi (64). If this is the case, then

the change from smooth to rough form might be an example of the transition from bacteria to fungi.

Biochemical Characteristics of the Diphtheroids--The organisms of Groups I and II have, in general, a much higher fermentative ability than do the known Actinomyces sp. (Tables 9, 10). On the basis of the fermentation of xylose, adonitol, glycerol, sorbose, and sodium pyruvate, the unknown isolates are different from any of the known species in Table 9. The isolates of Groups I and II (Tables 10, 11) have the following characteristics in common with A. Suis as described by Grasser (29):

1) Summary of tests for acid production from carbohydrates:

Carbohydrate	Isolates (8 cultures) No. positive	A. suis (10 cultures) No. positive
Glucose	8	10
Mannitol	7	5
Glycerol	8	7
Xylose	8	10
Insoluble starch	8	10*
Soluble starch	7	
Raffinose	7	10
Adonitol	8	7
Arabinose	8	9
Galactose	8	10
Rhamnose	3	5
Lactose	3	10
Melibiose	7	10
Saccharose	6	9
Inulin	7	9
Dulcitol	6	1
Salicin	5	10
Sorbose	5	7

*Type of starch not given.

2) Oxygen requirements--facultative. The cultures will grow equally well aerobically, microaerophilically, or anaerobically.

Table 9. Biochemical Reactions of Known Diphtheroid Cultures

	Catalase									O_2 require				NO_3	Indole	MR	VP	H_2S	Starch hydrolysis						Gel.		Litmus milk					
	Microcolonies	Macro 7 day	Macro 14 day	Aerob slant	Aerob + CO_2	Anaerob	Anaerob + CO_2	A. broth	Thio + TST	H - N - CO_2	Aerobic	Aerobic + CO_2	Anaerobic	Anaerobic + CO_2					7 day	20 day	Sol. 3 day	Sol. 7 day	Sol. 14 day	Insol. 3 day	Insol. 7 day	Insol. 14 day	Acid fast	7 day	14 day	Acid	Alkaline	A. coag.	Alk. coag.	Peptoniz.	Reduction
A. bovis	-	-	-	-	-	-	-	-	-	+	0	2	2	2	-	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+
A. naeslundii	-	-	-	-	-	-	-	-	-	-	2	3	2	2	+	-	+	-	W	W	+	+	+	+	W	W	-	-	-	-	+	-	+	+
A. israelii	+		+		-	-	-	-	-	+	0	2	3	2	+	-	-	+	-	+	No growth						-		-	+	-	-	-	+
A. eriksonii	-	-	-	-	-	-	-	-	-	-	0	0	2	2	-	-	+	+	W	W	-	-	-	+	+		-	-	-	-	+	-	-	+
A. odontolyticus	-	-	-	-	-	-	-	-	-	-	0	+	+	2	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
A. parabifidus	+	+	+	+	+	+	+	+	+	+	0	2	3	2	+	-	+	+	-	+	-	-	-	-	+	+	-	-	-	-	-	+	+	+
A. propionicus	-	-	-	-	+	-	-	-	-	-	2	2	2	2	+	-	-	-	-	W	-	-	-	-	-	+	-	+	+	-	+	-	W	+
A. discofoliatus	+	+	+	+	+	+	+	+	-	-	0	2	3	3	-	-	-	-	-	-	No growth						-	-	-	+	-	-	-	+
N. dentocariosus	+			-	+	-	-	+	-	-	3	2	0	0	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+
O. viscosus	+	-	-	+	-	-	-	-	-	-	0	2	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
R. pleuriticum	+	-	-	-	-	-	-	-	-	+	0	0	2	2	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+
A. suis											+	+	+																					

Table 9. (continued)

	Fermentation reactions of known cultures											
	A. bovis	A. naeslundii	A. israelii	A. eriksonii	A. odontolyticus	A. parabifidus	A. propionicus	A. viscofoliatus	N. dentocariosus	O. viscosus	R. pleuriticum	A. suis*
Glucose	5	5	7	5	7	5	7	-	7	-	-	+
Mannitol	-	-	-	7	-	14	7	-	-	-	-	50%
Glycerol	-	-	-	-	-	7	-	-	-	-	-	70%
Xylose	-	-	21	5	-	5	-	-	-	-	-	+
Insol. starch	7	+	21	7	21	-	-	21	-	21	-	
Raffinose	-	7	7	7	-	-	7		-	-	-	+
Adonitol	-	-	-	-	-	7	21	-	-	-	-	70%
Arabinose	-	-	21	5	-	-	-	-	-	-	-	90%
Fructose	10	5	7	5	7	5	7	7	7	-	-	
Galactose	5	5	21	5	-	5	7	-	-	-	-	+
Mannose	14	5	21	5	-	5	7	21	7	-	-	
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	50%
Ribose	-	-		5		5			-	-	-	
Sorbose	-	-	-	-	-	-	-	-	-	-	-	
Cellobiose	-	21	7	5	-	-	-	-	-	-	-	
Lactose	5	21	7	5	14	5	7	7	-	-	-	+
Maltose	5	5	7	5	-	-	7	7	7	-	-	+
Melibiose	-	5	7	5	-	5	-	-	-	-	-	
Sucrose	5	5	7	5	7	5	7		7	-	-	90%
Trehalose	-	5	7	5	-	-	7	-	7	-	-	+
Melezitose	-	-	-	14	-	-	-	-	-	-	-	
Dextrin	7	14	14	7	21	-	-	-	-	21	-	
Glycogen	7	-	-	7	21	-	-	-	-	-	-	
Inulin	-	14	-	-	-	-	-	-	-	-	-	90%
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	10%
Inositol	7	7	-	-	-		-	-	-	-	-	
Salicin	-	7	7	7	-	-	-	-	-	-	-	+
Sorbitol	-	-	-	-	-	-	7	-		-	-	20%
Lactic acid	-	-	-	-	-	-	-	-	-	-	-	
Sodium acetate	-	-	-	-	-	-	-	-	-	-	-	
Sodium pyruvate	-	-	-	-	-	-	-	-	-	-	-	
Soluble starch	7	14	-	7	-	-	-	-	-	-	-	
Starch												+

*Results from 1962 paper by Grasser (29). Percent from 10 cultures.

Table 9. (continued)

Key to Tables 9, 10, 11, 12, 13, 14, 15

Fermentation reactions:

The numbers refer to the number of days after inoculations before acid was observed. Negative means that no acid was produced in 21 days.

Oxygen requirements:

Amount of growth is recorded from 0 (no growth) to 4 (maximum growth).

Colonial morphology:

Relative size of colonies on BHI slants used in the test for oxygen requirements. F--very small (fine) colonies; M--medium size colonies; L--large colonies.

Source:

SU--swine udder

BJ--beef jowl

BL--beef liver

SJ--swine jowl

W--weak reaction.

Table 10. Biochemical Reactions of Eight Selected Unknown Diphtheroid Cultures

Culture No.	Catalase				O_2 Req.							Starch hydrol.			Litmus milk									Hemolysis	Acid fast
	BHI broth TTST	BHI Agar Anaerobic	Aerobic	Microaero.	Blood Aerobic	Blood Microaero.	Blood Anaerobic	BHI Aerobic	BHI Aerobic + CO_2	BHI Anaerobic	Nitrate	Indole	Methy red	Voges Proskauer	Hydrogen sulfide	14 day soluble	Insoluble	Gelatin	Acid	Alkaline	A. coag.	Alk. coag.	Peptonized	Reduction		
25	-	-	-	-	3	2	2	2	2	1	-	-	-	-	-	-	w	-	+	-	+	-	+	w	-	-
43	-	-	-	-	3	3	2	2	2	2	-	-	-	-	-	-	-	-	+	-	+	-	+	w	+	-
94	-	-	-	-	3	2	3	2	2	1	+	-	-	-	-	-	w	-	+	-	+	-	+	w	+	-
108	-	-	-	-	3	2	2	2	2	1	+	-	-	-	-	-	w	-	+	-	+	-	+	w	+	-
126	-	-	-	-	3	3	2	3	3	3	-	-	-	-	-	-	w	-	+	-	+	-	+	w	+	-
230	-	-	-	-	2	2	2	2	2	2	+	-	-	-	-	-	-	-	-	-	-	-	-	w	-	-
271	-	-	-	-	3	3	2	3	3	3	-	-	-	-	-	-	w	-	+	-	+	-	+	w	+	-
326	-	-	-	-	2	3	2	2	2	2	+	-	-	-	-	-	w	-	+	-	+	-	_	w	+	-

Table 10. (continued)

	Fermentation reactions							
	25	43	94	108	126	230	271	326
Glucose	14 7	14 -	14 14	14 14	14 14	7 7	14 7	21 14
Mannitol	14 14	- 14	14 14	14 14	14 14	7 14	- -	14 14
Glycerol	14 -	14 21	14 21	14 21	14 -	7 14	21 -	14 -
Xylose	14 14	14 7	14 14	14 -	21 14	7 7	14 14	14 14
Ins. starch	14 -	14 -	14 21	21 21	14 21	14 -	21 21	21 21
Raffinose	14 14	- 14	14 -	14 21	- -	7 14	14 14	14 14
Adonitol	7 14	14 14	14 14	- 14	- 21	7 14	14 -	7 14
Arabinose	14	21	21	21	21	7	14	14
Fructose	7	14	14	14	14	7	-	21
Galactose	14	21	21	14	14	7	14	7
Mannose	14	14	-	14	14	7	-	14
Rhamnose	-	-	-	14	-	14	-	21
Ribose	7	14	-	14	-	7	7	7
Sorbose	14	14	14	14	14	14	14	14
Cellobiose	-	14	21	14	21	7	21	14
Lactose	14	14	-	-	-	7	-	-
Maltose	14	14	14	14	14	-	-	14
Melibiose	7	14	14	14	-	7	7	14
Sucrose	-	21	-	14	-	7	-	14
Trehalose	-	14	-	-	14	14	-	14
Melezitose	14	-	-	-	14	14	-	14
Dextrin	14	14	14	14	14	14	-	14
Glycogen	-	7	14	14	14	14	14	14
Inulin	14	14	14	14	14	14	-	14
Dulcitol	14	14	-	14	14	14	-	21
Inositol	14	14	14	14	14	14	14	14
Salicin	-	14	14	14	14	7	-	-
Sorbitol	14	-	-	14	14	14	-	21
Lactic Acid	-	-	-	-	-	-	-	-
Sod. pyruvate	7	7	7	7	7	7	7	7
Sol. starch	14	14	21	14	14	-	21	21
Sod. acetate	14	-	14	14	-	-	-	14

Table 11. Biochemical Reactions of Group I Diphtheroids

Culture no.	Source	Catalase: TTST BHI broth	Catalase: BHI Agar: Aerobic	Catalase: BHI Agar: Anaerobic	Catalase: BHI Agar: Microaero.	Nitrate	Indole	Methyl red	Voges-Prosquar	H_2S: 7 day	H_2S: 14 day	Hemolysis	O_2 requirements: Blood: Aerobic	Blood: Anaerobic	Blood: Micro.	O_2 requirements: BHI: Aerobic	BHI: Anaerobic	BHI: Micro.	Acid fast	Colonial Morphology: BHI: Aerobic	Colonial Morphology: BHI: Anaerobic	Colonial Morphology: BHI: Micro.	Fermentations: Glucose	Mannitol	Glycerol	Xylose	Raffinose	Adonitol	Insol. starch
230	SU	-	-	-	-	-	-	+	-	-	-	-	2	2	2	2	2	2	-	L	L	M	7	7	7	7	7	7	14
327	SU	-	-	-	-	-	-	-	-	-	-	+	2	2	2	2	2	3	-	F	F	F	7	7	7	7	7	7	7
333	SU	-	-	-	-	+	-	+	-	-	-	-	2	2	2	2	2	2	-	F	L	M	7	7	7	7	14	7	14
179	SJ	-	-	-	-	-	-	-	-	+	+	+	3	2	3	2	2	2	-	F	F	F	7	14	14	7	14	7	7
24	SJ	-	-	-	-	-	-	-	-	-	-	+	2	2	2	2	2	2	-	F	L	M	7	7	7	7	7	7	14
144	SL	-	-	-	-	-	-	-	-	-	-	+	3	2	2	1	2	2	-	F	F	F	7	14	7	14	14	14	7
130	BL	-	+	-	-	-	-	-	-	-	-	+	1	2	2	1	2	2	-	F	F	M	14	-	14	-	-	14	-

Table 12. Biochemical Reactions of Group II Diphtheroids

Culture no.	Source	Catalase: Thio BHI broth	Catalase: BHI Agar: Aerobic	Catalase: BHI Agar: Anaerobic	Catalase: BHI Agar: Microaero.	Nitrate	Indole	Methyl red	Voges-Proskaer	Hydrogen sulfide	Hemolysis	O_2 requirements: Blood: Aerobic	Blood: Anaerobic	Blood: Micro.	BHI: Aerobic	BHI: Anaerobic	BHI: Micro.	Acid fast	Colon. Morph. BHI: Aerobic	Colon. Morph. BHI: Anaerobic	Colon. Morph. BHI: Micro.	Fermentations: Glucose	Mannitol	Glycerol	Xylose	Raffinose	Adonitol	Insol. starch
49	SU	-	-	+	-	-	-	-	-	+	+	2	3	2	2	2	2	-	F	F	F	7	14	7	7	14	7	14
51	SU	-	+	-	-	-	-	-	-	+	+	2	3	2	1	1	2	-	F	F	F	7	14	7	7	14	7	14
52	SU	-	-	-	-	-	-	-	-	-	-	2	3	2	2	2	2	-	F	F	F	-	-	-	-	-	-	-
103	SU	-	-	-	-	-	-	-	-	±	-	2	2	2	1	1	1	-	F	F	F	14	14	-	14	7	-	-
106	SU	-	-	-	-	-	-	-	-	-	+	2	3	2	1	1	1	-	F	F	F	14	-	-	21	-	-	-
111	SU	-	-	-	-	-	-	-	-	-	+	3	3	3	2	2	2	-	F	M	M	-	-	-	-	-	-	-
167	SU	-	-	-	-	-	-	-	-	-	+	3	3	2	1	1	1	-	F	F	F	-	-	-	-	-	-	-
206	SU	-	-	-	-	-	-	-	-	-	+	3	3	2	2	2	2	-	F	F	F	21	14	-	21	-	-	21
209	SU	-	-	+	-	+	-	+	+	-	+	3	2	2	1	1	1	-	F	F	F	14	14	-	14	14	14	14
233	SU	-	-	-	-	-	-	+	-	-	-	3	4	2	3	3	3	-	F	F	F	14	14	14	14	14	-	14
287	SU	-	-	-	-	-	-	+	-	-	+	3	2	2	2	2	2	-	F	F	F	7	-	14	14	14	14	-
289	SU	-	-	-	-	-	-	-	-	-	+	3	3	2	3	3	3	-	F	F	F	14	14	-	14	14	-	-
304	SU	-	-	-	-	-	-	-	-	-	-	2	2	2	2	2	2	-	F	F	F	-	-	-	-	-	-	-
325	SU	-	-	-	-	-	-	-	-	-	+	2	3	2	3	3	3	-	F	F	F	-	14	-	-	-	-	-
13	SJ	-	-	-	-	-	-	-	-	-	+	2	3	3	2	2	2	-	F	F	F	14	-	-	-	-	-	-
18	SU	-	-	-	-	-	-	-	-	-	+	2	2	2	2	2	2	-	F	F	F	14	14	-	14	7	14	-
178	SU	-	-	-	-	-	-	-	-	-	-	0	0	2	0	0	1	-	F	F	F	7	14	14	14	14	-	-
243	SL	-	-	-	-	-	-	-	-	±	+	2	3	2	1	1	1	-	F	F	F	14	14	-	14	14	14	14
75	BL	-	-	-	-	-	-	+	-	-	-	3	3	2	2	2	2	-	F	F	L	14	14	14	21	14	-	14

Table 12. (continued)

Culture no.	Source	Catalase BHI: Thio	BHI broth	Aerobic	Anaerobic	Microaero.	Nitrate	Indole	Methyl red	Voges-Proskaer	H_2S: 7 day	14 day	Hemolysis	O_2 requirements Blood: Aerobic	Anaerobic	Micro.	O_2 requirements BHI: Aerobic	Anaerobic	Micro.	Acid fast	Colon. Morph. BHI: Aerobic	Anaerobic	Micro.	Fermentations: Glucose	Mannitol	Glycerol	Xylose	Raffinose	Adonitol	Insol. starch
270	SU	-	-	-	-	-	-	-	-	-	-	-	+	3	2	2	1	1	1	-	F	F	F	21	-	-	-	-	-	-
273	SU	-	-	-	-	-	-	-	-	-	-	-	+	2	3	2	2	2	2	-	F	F	F	14	14	14	-	14	14	-
322	SU	-	-	-	-	-	-	-	-	-	-	-	+	2	3	2	3	3	3	-	F	F	F	-	-	-	-	-	-	-
241	SJ	-	-	-	-	-	-	-	-	-	-	-	+	3	3	2	2	2	2	-	F	F	F	7	14	14	14	-	-	14
218	BL	-	-	-	-	-	-	-	-	-	-	-	-	3	2	2	2	2	2	-	F	F	F	-	-	-	-	-	-	-
255	BL	-	-	-	-	-	-	-	+	-	-	-	+	3	3	4	2	2	2	-	F	F	F	14	-	14	7	14	14	-
175	SJ	-	-	-	-	-	+	-	+	-	+	+	+	2	2	3	1	1	1	-	M	L	M	7	-	14	7	-	7	14
253	BL	-	-	+	-	-	-	-	+	-	-	-	-	3	3	4	3	3	3	-	F	F	F	14	21	14	14	14	-	-
42	SU	-	-	-	-	-	-	-	-	-	-	-	+	2	2	3	1	1	1	-	F	F	F	-	-	14	-	-	-	-
79	BL	-	-	-	-	-	-	-	-	-	-	-	+	3	2	4	1	1	1	-	F	F	L	14	-	-	-	14	-	-

Table 13. Biochemical Reactions of Group III

Culture no.	Source	Catalase: Thio BHI broth	Catalase: BHI Agar: Aerobic	Catalase: BHI Agar: Anaerobic	Catalase: BHI Agar: Microaero.	Nitrate	Indole	Methyl red	Voges-Proskauer	Hydrogen sulfide	Hemolysis	O_2 requirements: Blood: Aerobic	Blood: Anaerobic	Blood: Micro.	BHI: Aerobic	BHI: Anaerobic	BHI: Micro.	Acid fast	Colon. Morph.: Aerobic	Colon. Morph.: Anaerobic	Colon. Morph.: Micro.	Fermentations: Glucose	Mannitol	Glycerol	Xylose	Raffinose	Adonitol	Insol. starch
168	SU	-	-	-	+	-	+	+	+	-	-	-	2	3	2	1	1	1	-	F	F	F	7	7	7	14	-	7	-
221	BL	-	-	-	+	-	+	+	+	-	-	-	1	3	2	1	1	1	-	F	F	F	7	7	7	14	-	14	-
288	BL	-	-	-	+	-	+	+	+	-	-	-	2	2	2	1	1	1	-	F	F	F	7	7	7	7	-	14	-

not ferment raffinose or insoluble starch. The only precipitin line demonstrated with Group III was against A. odontolyticus. However, Group III varied markedly from A. odontolyticus in its biochemical characteristics.

Group IV (Table 14) contains 3 organisms which demonstrated precipitin lines with both A. suis and with the known Actinomyces spp. This group is biochemically characteristic of A. suis.

The 9 isolates of Group V (Table 15) showed no antigenic relationship with any of the organisms tested. Group V isolates grew best under anaerobic conditions and had reduced fermentative activity.

Table 14. Biochemical Reactions of Group IV Diphtheroids

Culture no.	Source	Catalase: Thio BHI broth	Catalase: BHI Agar Aerobic	Catalase: BHI Agar Anaerobic	Catalase: BHI Agar Microaero.	Nitrate	Indole	Methyl red	Voges Proskauer	Hydrogen sulfide	Hemolysis	O_2 requirements: Blood Aerobic	Blood Anaerobic	Blood Micro.	BHI Aerobic	BHI Anaerobic	BHI Micro.	Acid fast	Colon. Morph.: Aerobic	Colon. Morph.: Anaerobic	Colon. Morph.: Micro.	Fermentations: Glucose	Mannitol	Glycerol	Xylose	Raffinose	Adonitol	Insol. starch
107	SU	-	-	-	-	-	-	-	-	-	+	0	2	1	1	1	1	-	F	F	F	7	7	7	21	7	14	14
329	SU	-	-	-	-	-	-	-	-	-	+	2	2	2	2	2	2	-	F	F	F	7	7	7	7	7	7	14
142	SL	-	-	-	-	-	+	+	-	-	+	3	1	2	1	3	2	-	F	M	L	7	14	7	7	7	7	14
80	BL	+	+	-	+	-	+	+	+	-	-	0	2	0	1	3	1	-	F	F	F	7	-	7	-	14	14	-
122	BL	-	-	-	-	-	-	-	-	-	-	3	3	3	2	2	2	-	F	F	F	14	-	-	-	14	-	-
194	BL	-	-	-	-	-	-	-	-	-	-	0	1	0	1	1	1	-	F	F	F	-	-	-	-	-	-	-
234	SU	-	-	-	-	-	-	-	-	-	-	0	1	0	1	1	1	-	F	F	F	-	-	-	-	-	-	-
117	BL	-	-	-	-	-	-	-	-	-	-	0	1	1	1	1	1	-	F	F	F	-	-	-	-	-	-	-

Table 15. Biochemical Reactions of Group V Diphtheroids

Culture no.	Source	Catalase: Thio BHI broth	Catalase: BHI Aerobic	Catalase: BHI Anaerobic	Catalase: BHI Microaero.	Nitrate	Indole	Methyl red	Voges-Proskauer	Hydrogen sulfide	Hemolysis	O_2 requirements: Blood Aerobic	Blood Anaerobic	Blood Micro.	BHI Aerobic	BHI Anaerobic	BHI Micro.	Acid fast	Colon. Morph.: Aerobic	Colon. Morph.: Anaerobic	Colon. Morph.: Micro.	Fermentations: Glucose	Mannitol	Glycerol	Xylose	Raffinose	Adonitol	Insol. starch
217	BL	-	-	-	+	-	+	-	-	+	-	3	3	3	1	1	1	-	F	F	F	7	-	21	14	-	14	14
279	SJ	-	-	-	-	-	+	-	-	-	-	3	3	3	2	1	1	-	M	M	M	7	14	14	14	-	14	14
303	SU	-	-	-	-	-	-	+	-	±	-	2	2	2	3	1	2	-	L	L	L	7	14	7	14	14	7	7

Isolations from Miscellaneous Abscesses

The organisms isolated from miscellaneous abscesses (Table 16) were characteristic of the etiological agents already described. The only significant correlation observed within this group was the presence of bacteroides in 14 of the 18 abscesses examined.

Table 16. Isolations from Miscellaneous Abscesses

Source	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis*
Swine belly fat	Streptococci	5.0	thick	green	none	clear
	Diphtheroid 178	5.0	thick	green	none	clear
Swine belly fat	Diphtheroid 179	2.5	thick	green	none	none
	bacteroides	2.5	thick	green	none	none
Swine stomach membrane	bacteroides	3.7	thick	green	none	none
Swine stomach membrane	*E. coli*	1.2	thick	green	none	none
Swine peritoneal cavity	bacteroides	3.7	granular	reddish	none	none
Swine peritoneal cavity	bacteroides	2.5	thick	yellowish	none	clear
Swine peritoneal cavity	bacteroides	2.5	thick	green	none	clear
Swine rib cage	*Streptococcus*	3.7	thick	green	none	clear
Swine rib cage	Diphtheroid 18	5.0	thick	green	none	clear
Swine liver	bacteroides	2.5	creamy	white	none	none
Swine liver	bacteroides	2.5	thick	green	none	clear
Swine lung	Diphtheroid 142	2.5	thick	tan	none	none
	bacteroides	2.5	thick	tan	none	none
Swine lung	Diphtheroid 144	2.5	thick	tan	none	none
	bacteroides	2.5	thick	tan	none	none

Table 16. (continued)

Source	Name	Abscess size (cm)	Exudate consistency	Exudate color	Odor	Hemolysis*
Swine lung	Diphtheroid 243	0.6	thick	tan	none	none
	bacteroides	0.6	thick	tan	none	none
Beef stomach wall	bacteroides	1.2	thick	white	none	none
Beef stomach wall	bacteroides	1.2	thick	green	none	none
Beef stomach wall	bacteroides	1.2	thick	green	none	none
Beef somach wall	bacteroides	1.2	thick	green	none	clear

*Determined by subculture on blood agar.

Topics for Further Study--During this study it became evident that further study is needed to determine:

1) the bacterial relationship of individual abscesses manifested in different tissues within an animal.

2) the relationship of liver flukes to beef liver abscesses. They might act as carriers of the bacteria causing the abscess or they might predispose the infection by causing necrotic tissue.

3) whether high incidences of abscesses are associated with particular feed lots and/or with particular herds.

4) if cattle and swine raised in close proximity develop abscesses containing related organisms.

5) the relationship of abscesses to the type of feed and the methods of feeding.

6) whether Koch's postulates can be carried out with the diphtheroids.

7) the relationship of the Corynebacterium sp., Odontomyces viscosus, and the Actinomyces sp. These studies are needed because:

A) the main characteristics used for differentiation of these organisms, other than antigenic differentiation, are catalase production and proteolysis. These traits are not stable. This is particularly significant because these organisms are in two separate orders and some of the organisms of one order, such as Corynebacterium hemolyticum and Corynebacterium pyogenes, have as many

characteristics in common with the genus Actinomyces of the order Actinomycetales as they do with the genus Corynebacterium of the order Eubacteriales.

B) only one antigenic component (water soluble, acetone insoluble antigen which must be excreted by the cell) has been used for serological studies. This test has worked well for differentiation but does not show the antigenic relationship of the organisms.

8) the significance of the change from a bacterial form to a filamentous form as demonstrated by the diphtheroids. This study may be of importance because:

A) if the report by Grasser (29) is correct, that fermentative ability varies with the morphology, it may explain the variations in biochemical activities reported for the diphtheroids. It might also raise some additional questions on classification, especially if this variation occurs in proteolytic ability or in the ability to produce catalase.

B) if it can be substantiated that a change in the oxygen tolerance of Actinomyces israelii occurred with a change from rough to smooth form (62), it would open some questions in regard to the classification of the diphtheroids by their oxygen requirements.

C) it was observed in this laboratory that the cell walls of A. bovis, which are generally in the bacterial form, can be quite readily broken by using lysozyme and the French

pressure cell. This is in contrast to the cell walls of A. israelii, which is generally in the filamentous form, which cannot readily be broken using this technique or any other technique which has been reported. This raises the question whether A. israelii cell walls could be broken in the bacterial form. If lysis of the cells could be carried out in this manner, it would solve the problem of obtaining intact DNA for base ratio studies. If the structure of the cell wall is changed, it would raise questions in regard to cell wall analyses.

D) all of these possibilities raise interesting questions about the biochemical and genetic mechanisms of the change, especially since the Actinomycetales are considered to be phylogenetically between the bacteria and the fungi (64) and this might be an example of the transition between the two.

9) the importance of spherical bodies in the diphtheroids.

10) what these variations in the diphtheroids may mean in regard to pathogenicity, host specificity, and interrelationship with the unclassified, nonpathogenic diphtheroids.

CONCLUSIONS

Abscesses of beef livers were most commonly associated with bacteroides isolates (88.4%). Diphtheroids were isolated in 16% of the beef liver abscesses, but they were found in pure culture in only 6.3% of the cases. These results are consistent with the findings of previous investigators. One of the diphtheroids was similar to Actinomyces suis; no other reports of isolation of this organism from beef liver abscesses could be found in the literature.

Actinobacillus lignieresii, the commonly reported causative agent of abscesses of the soft tissue of beef jowls, was found in 68.2% of the beef jowl abscesses which were examined.

Although streptococci were not often reported in the literature as the causative agents of beef jowl abscesses, streptococci were found in pure culture in 27.2% of the abscesses. This may indicate that the streptococci are important in beef jowl abscesses.

Streptococci were isolated from the majority (70.9%) of swine jowl abscesses. This supports reports of previous work in which streptococci were reported as the main causative agents of swine jowl abscesses.

The use of anaerobic isolation techniques facilitated the isolation of bacteroides from 14.5% of the abscesses. In comparison, Collier (13) reported isolation of one of the bacteroides, Sphaerophorus necrophorus, from only 0.41% of his samples when he used random anaerobic sampling techniques in conjunction with aerobic methods. Obligate anaerobes are, therefore, more important in the etiology of

swine jowl abscesses than was previously reported. Organisms similar to Actinomyces suis were found in two swine jowl abscesses. No other report of its presence in this type of abscess could be located.

Four groups of organisms were associated with swine udder abscesses (diphtheroids, 51.4%; bacteroides, 32.8%; streptococci, 21.3%; and Enterobacteriaceae, 18.0%). A. suis-like organisms were isolated from 3 of the swine udder abscesses. These isolates were identical in their biochemical reactions with those described by Grasser (29) in East Germany.

Bacteroides were isolated from the majority of abscessed tissues other than beef livers, beef jowls, swine jowls, and swine udders. An abscess from one of the swine livers did contain an A. suis-like organism. This is the only reported isolation of this organism from a swine liver.

The 65 diphtheroids which were isolated were placed in 5 serological groups. Group I contained 7 organisms characteristic of A. suis. Group II contained isolates which were characteristic of Corynebacterium pyogenes. Group III contained 3 organisms which were antigenically similar to, but biochemically different from, Actinomyces odontolyticus.

Group IV organisms were biochemically similar to A. suis and cross reacted with known Actinomyces sp. and with some organisms in Groups I, II, and III.

Antigens from Group V organisms did not react with any of the antisera. These organisms grew best under anaerobic conditions and were less fermentative than the other organisms. Genus and species designation for these organisms will require further study.

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