THE SOURCE OF INFECTION IN ACTINOMYCOSIS[1]

JOHN SLACK[2]

Department of Bacteriology and Immunology, University of Minnesota, Minneapolis

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There are two theories regarding the source of infection in actinomycosis. The exogenous theory introduced by Bostroem (1891) was based upon the fact that he observed awns of grass or grain in the actinomycotic lesions of man and cattle. This theory has been propagated and enlarged upon by numerous authors but has gained support mainly from clinical observations.

The endogenous theory did not receive recognition until relatively recent times, although supporting evidence can be found throughout the literature on actinomycosis. Lord (1910) noticed the occasional presence of organisms in sputum having the morphology of actinomycetes. By means of smears and sections, he also demonstrated actinomycetes in the contents of 16 carious teeth. Naeslund (1925) cultivated anaerobic actinomycetes from the oral cavity and stated that these organisms were nearly identical culturally with the anaerobic forms cultivated from cases of actinomycosis. Naeslund (1931) was unsuccessful in his attempt to produce experimental actinomycosis in animals with strains of anaerobic actinomycetes isolated from the oral cavity. Emmons (1935) cultivated anaerobic strains of actinomycetes from the oral cavity which resembled both culturally and morphologically the typical *Actinomyces bovis*. In 1936 and 1938 he cultured tonsils and obtained several strains of actinomycetes. These tonsillar strains were avirulent for guinea pigs. Lord and Trevett (1936) isolated 4 strains of anaerobic actino-

[1] Summary of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota.

[2] Now of the Department of Pathology and Bacteriology, University of Nebraska Medical School, Omaha, Nebraska.

mycetes from the dental scum and contents of carious teeth of 90 patients.

ACTINOMYCETES IN THE TONSILS

Ruge (1896) was the first to record the presence of actinomyces-like granules in the crypts of tonsils. Jonathan Wright (1904) found one actinomyces granule in sections from 75 tonsils. Wilkinson (1928) examined 10,000 pairs of tonsils and found actinomyces-like granules in the crypts of 177 of the tonsils. Davis (1914) found granules in 30 out of 122 pairs of tonsils. Microscopically, these presented a ray-like structure which resembled a test-tube brush. Smears from the granules showed unbranched filaments, cocci, fusiform organisms and large spirochetes. Cultures were negative for actinomycetes. Pilot and Davis (1918) and Davis and Hall (1924) reconfirmed the above results and state that in no single instance was a true actinomyces found in the crypts. Naeslund (1925) was the first to show by cultural means that anaerobic species of actinomycetes existed in tonsils.

Tunnicliff (1926) isolated from an actinomyces-like tonsillar granule a weakly gram-positive, motile anaerobic rod which in pure culture produced rosettes and test-tube-brushlike forms. Tunnicliff and Jackson (1930) isolated a second strain of this organism from a tonsil granule and called it *Vibriothrix tonsillaris*.

PERSONAL INVESTIGATION

One hundred pairs of tonsils from routine tonsillectomies were secured from the Minneapolis General Hospital. They were taken from two distinct age groups, 50 pairs from children 1 to 15 years old, and 50 pairs from adults 15 to 63 years old. All of the patients were from Minneapolis.

Following the tonsillectomy, the tonsils were placed in sterile vials and immediately taken to the laboratory. At the laboratory they were transferred to sterile Petri dishes and then several transverse sections were cut with a sterile razor blade. They were so sliced because in many of the tonsils the crypts were almost completely closed by epithelial overgrowths and could not be washed out. Following this, the crypts were washed out by

squirting saline into them by means of a capillary pipette on the end of which was a rubber bulb. In this manner all the debris and caseous plugs were removed from the crypts. Then a portion of each tonsil, which had not been washed out, was placed in 4 per cent formalin for fixing and subsequent sectioning. The caseous plugs varied in size from microscopic to 1–3 mm. in diameter. They were grayish-yellow, cheesy, round or lobulated, foul-smelling masses. The plugs were present in over 90 per cent of the tonsils, although in a few instances none could be found even after careful dissection.

For the microscopic examination, one or more of the plugs were placed on a slide and crushed under a cover-slip. In these wet preparations, the majority of the plugs appeared to be made up of filamentous organisms whose formation sometimes resembled that of a test-tube brush. However, when the cover-slips were removed and the plugs were smeared and stained with Gram's stain, only masses of cocci, fusiform bacilli and spirochetes were observed. In only one instance was a true branching filamentous actinomyces found.

For cultivation, all of the caseous plugs and debris were crushed and emulsified in saline. Then 1.1 ml. of this turbid solution was pipetted off and distributed as follows: 0.1 ml. was inoculated on Sabouraud's slant for Monilias, 0.5 ml. was inoculated into a tube of melted 1 per cent glucose veal-infusion agar (pH 7.4) and the remaining 0.5 ml. was inoculated into melted veal-infusion agar without glucose (pH 7.4). From these, three dilution tubes of the corresponding media were inoculated with a platinum loop. The purpose of the veal agar with and without glucose was to determine whether glucose was necessary for the growth of these organisms.

The cultures were incubated at 37°C. for three weeks and then examined. Tubes in which the colonies were separated sufficiently to facilitate their removal were nicked with a triangular file, placed in 70 per cent alcohol for 5 minutes and then cracked by heat. The agar column was pushed out into a sterile Petri dish with a glass rod. These agar columns were then examined with a dissecting microscope and when a well isolated colony was

found, a stiff wire was used to dig through the agar down to the colony. Then a small portion of the colony was removed, smeared, stained by Gram's method, and if this revealed an actinomyces, subcultures were made.

Colonies of anaerobic actinomycetes were found in 14 (14 per cent) of the primary mixed cultures and pure cultures were isolated from 11 (11 per cent) of the tonsils. Five of these were from children and six were from adults; thus there was no selection as far as age was concerned. *Monilia albicans* was isolated from 12 per cent of the tonsils.

These organisms grew well in veal-infusion agar either with or without glucose, although a more abundant growth occurred in the glucose agar.

Typical "zone growth" was observed in all instances. By this I mean that no growth occurred in the upper 1–2 cm. of agar, then there was a dense area of punctiform colonies, and below these, scattered individual colonies occurred throughout the remainder of the medium. These colonies varied from 1–3 mm. in diameter, were grayish white, lobed, and either butyrous or finely granular. One is unable to differentiate them from colonies of streptococci or staphylococci, except when mycelial elements radiate out from the colony into the surrounding agar. These organisms showed no tendency to change their oxygen requirements and were, without exception, lost on the 2nd, 3rd, or 4th subculture. This took place in spite of all attempts to provide enriched media such as ascitic fluid, blood or serum agar, or by varying the time of subculturing anywhere from 5–21 days.

Microscopically, the diphtheroid form was the most common. These structures varied from 1.5μ to 15μ in length and from 0.6μ to 1μ in width. They were granular, often clubbed and branched (fig. 1). The typical fine, frequently branched mycelium was limited almost entirely to young cultures, although at times it was seen in the older cultures. These actinomycetes were gram-positive, nonacidfast and did not form spores.

The portions of tonsils which had been fixed were removed, sectioned and stained with hematoxylin-eosin. It is to be noted that these pieces were random samples and that only a single

section was cut from each tonsil. Microscopically, structures of various types which resembled in some degree the sulfur granules of actinomycosis were found in the crypts of 31 tonsils. These can be classified as follows:

True Actinomyces granule	1
Vibriothrix tonsillaris	6
Amorphous masses of bacteria	24

As noted, a true actinomyces granule was found in only one tonsil. This granule was observed in an open crypt and there was

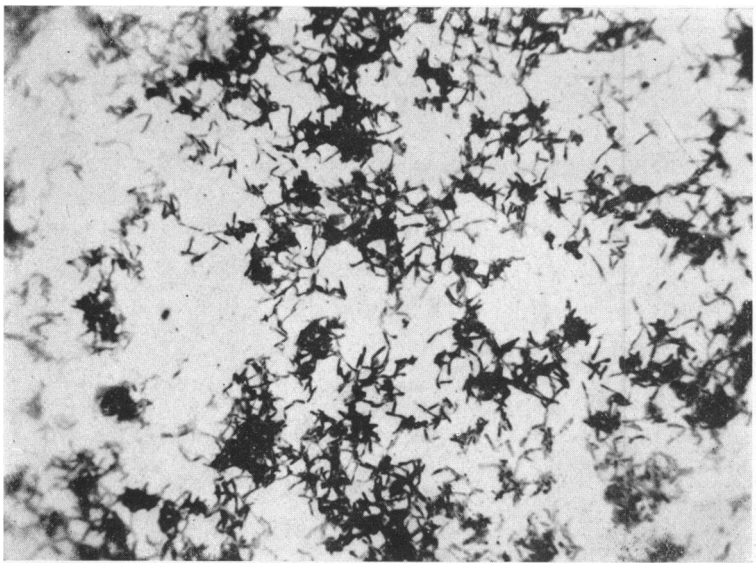

FIG. 1. DIPHTHEROID FORM OF ACTINOMYCES ISOLATED FROM TONSIL

no evidence of ulceration or tissue reaction. One hesitates to call this a sulfur granule because there was no pathology and the structure was not identical with that of a sulfur granule. There were no true hyalinized clubs at the periphery, although, with the oil-immersion, hyaline material could be seen. Also, the filaments extended out to the periphery of the granule. However, this alone would not prevent it from being classed as a true sulfur granule, because in the true granules there are usually areas where there are no clubs and then the filaments extend to the periphery.

However, if one accepts the idea that the club-formation in sulfur granules is the result of the tissue reaction and acts as a protective mechanism for the actinomyces, then it is possible that in the open crypts the actinomycetes do not need to form the protective hyaline clubs.

A pure culture of a microaerophilic actinomyces was obtained from this tonsil and direct smears from the washings showed branching actinomyces filaments. It was impossible to trace the

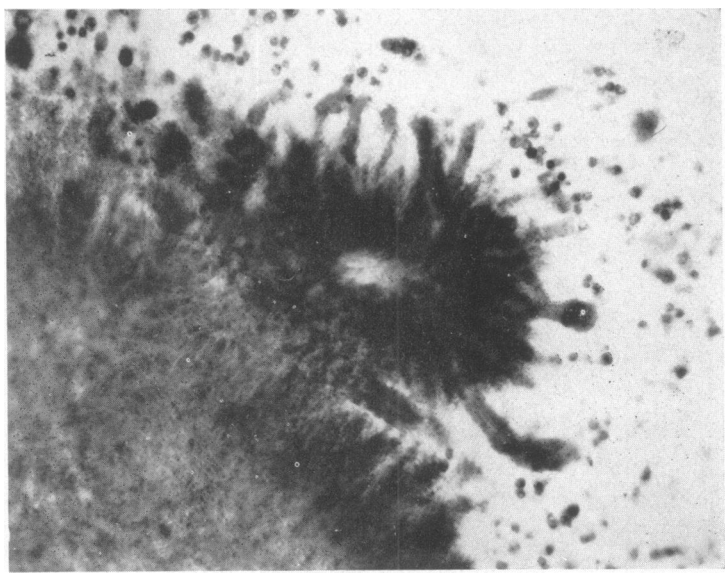

FIG. 2. MARGIN OF A GRANULE OF THE "TEST-TUBE BRUSH" ORGANISM (VIBRIOTHRIX TONSILLARIS) IN THE CRYPT OF A TONSIL

patient from whom the tonsils were removed, so it is not known if he ever developed actinomycosis.

Vibriothrix tonsillaris (fig. 2) has been described in Tunnicliff's paper. These organisms were found in the crypts of six tonsils and here again there was no evidence of ulceration or tissue reaction. The typical test-tube brush appearance is not always as clear-cut as it is in the above figure, but nevertheless, one should not confuse these with actinomyces granules.

The so-called "amorphous masses of bacteria" made up the largest group (34). These appeared in aggregates of varying

size and composition. Even with Gram-Weigert's stain it was impossible to determine the kinds of bacteria present, but it is most probable that there was a mixture of cocci, fusiform bacilli, and spirochetes.

ACTINOMYCETES IN PYORRHEA PUS

Lord (1910) found actinomycetes in carious teeth. Sullivan and Goldsworthy (1940) isolated 5 strains of anaerobic actinomycetes from 100 periodontal pockets and one strain from 24 carious teeth. These were compared with 5 strains isolated from cases of cervico-facial actinomycosis and found to be essentially identical. They studied fermentation reactions and found that all except one dysgonic strain fermented glucose, lactose, glycogen, salicin, sucrose, maltose, glycerol, mannitol, and dulcitol, producing acid but no gas. A number of animal inoculation experiments gave negative results. There is indeed a notable agreement between these results and the results obtained by Negroni and Bonfiglioli (1939).

PERSONAL INVESTIGATION

The gums of pyorrhea patients were squeezed until purulent exudate exuded and this was collected on a sterile swab. The swab was washed off in 1.5 ml. of sterile saline solution and then the vials were taken to the laboratory. There a Sabouraud's agar slant and the glucose veal-infusion and plain veal-infusion agar deeps were inoculated in the same manner and with the same amounts as described before.

Colonies of microaerophilic actinomycetes were found in 18 (18 per cent) of the primary mixed cultures and pure cultures were isolated from 12 (12 per cent) of the cases. These organisms were in every way identical with those isolated from tonsils. In cultures the "zone growth" was observed and morphologically the granular, sometimes clubbed, branched diphtheroid forms were the most common. Here again, except for one strain, all of the cultures died out on the 2nd, 3rd, or 4th subculture.

Monilia albicans was isolated from only one case. This is a very marked decrease from the 12 cultures obtained from tonsils.

There are two probable reasons for this: one is that the amount of material taken from the mouth was very small when compared to the washings of two tonsils. The other is that *Monilia albicans* does not find a suitable medium for growth in the purulent discharges of the pyorrhea pocket.

During the course of culturing these organisms it was customary to take an agar column which had numerous colonies growing in it and cut it into slices with a sterile wire. Each slice was transferred to a tube of ground liver broth and then rubbed against the side of the tube with a sterile metal spatula, the idea being that, if one colony would not grow, then possibly growth could be obtained by the simultaneous inoculation of several colonies.

These liver broth cultures were then placed in a McIntosh-Fildes jar and incubated at 37°C. under anaerobic conditions. After numerous failures, one strain (henceforth designated as D-14) finally grew. Strain D-14 was maintained for several generations on liver peptone broth and then, again using the McIntosh-Fildes jar, growth was obtained in glucose veal-infusion broth.

In broth, D-14 grew at the bottom of the tube and never produced turbidity. It usually formed compact, bread-crumb-like colonies, although sometimes a lattice-like growth occurred, and extended about 1 cm. up the sides of the tube. Occasionally fine mycelial filaments would radiate from the compact colonies, giving them a wooly appearance.

A strain of actinomyces (which will be referred to as "W") was isolated from a case of a cervico-facial actinomycosis which developed in a middle-aged woman following the extraction of some teeth. The exudate from facial sinuses was examined microscopically and cultured in glucose veal-infusion agar deeps. A pure culture of an anaerobic actinomyces was isolated from this material.

After strains D-14 and W had been maintained for several generations on glucose veal-infusion broth, various types of media were inoculated, placed in the McIntosh-Fildes jar and incubated at 37°C. The cultural characteristics of these two organisms are as follows:

	D-14	W
Litmus milk	Acid, no coagulation	Acid, no coagulation
Potato	Scant, adherent, grey spreading growth	Scattered, small, grey adherent colonies
Gelatin	Growth, no liquefaction	Growth, no liquefaction
Blood agar	Growth, no hemolysis	Growth, no hemolysis
Glucose, Maltose, Sucrose, Mannitol, Lactose	Acid, no gas	Acid, no gas
Indole	Negative	Negative
Voges-Proskauer	Negative	Negative

Thus the strain isolated from pyorrhea pus has the same cultural characteristics as the strain isolated from cervico-facial actinomycosis except that the latter did produce noticeably more acid in milk, and gave a positive methyl-red test in glucose broth.

These organisms were also tested for hemolysin by adding varying dilutions to a 2 per cent suspension of sheep red blood cells, incubating for 2 hours at 37°C., centrifuging and examining the sediment. This test was also negative.

ANIMAL INOCULATIONS

Numerous authors have inoculated various kinds of animals with pure cultures, sulfur granules or pus containing actinomycetes. Guinea pigs, rabbits, mice, cows, pigs, horses and dogs have been inoculated subcutaneously, intraperitoneally, intratesticularly, intracranially and into the anterior chamber of the eye, but the great majority of these experiments have been negative. These results indicate that the pathogenic actinomycetes have little or no invasive power for experimental animals.

Wolf and Israel (1891), James Wright (1905) and Lord (1910) inoculated rabbits and guinea pigs with actinomycetes and in a few instances obtained tumors which were histologically identical with actinomycotic tissue and which contained colonies of actinomycetes.

Naeslund (1929 and 1931) inoculated rabbits, guinea pigs, pigs, and cattle with pure cultures of actinomycetes isolated from human actinomycosis. All of the experiments were nega-

tive, except for one 8-month old heifer. This animal developed progressive actinomycotic lesions at two of the sites of inoculation after 3 weeks and one of the lesions discharged sulfur granules with clubs.

Mathieson, Harrison, Hammond and Henrici (1935) carried out a series of experiments on guinea pigs to determine if sensitization with anaerobic actinomycetes would render animals susceptible to the infection. In no case did the initial injection produce palpable masses or other evidences of infection. But all of the animals receiving repeated inoculations developed lesions of a type characteristic of actinomycosis. In four of the nine animals, the lesions contained typical sulfur granules. They conclude, "The results of these experiments strongly suggest that actinomycosis in man and animals does not result from a single invasion of the parasite, but that repeated exposure leading to sensitization is a factor in the etiology."

PERSONAL INVESTIGATION

The strains of actinomycetes which were isolated from tonsils were not adapted to growth in broth, thus the agar cultures had to be used for animal inoculations. An emulsion of agar and saline was prepared by grinding the agar columns in a mortar and pestle. This emulsion was easily drawn into a syringe and several guinea pigs were inoculated intraperitoneally. Some of the animals received 2 or 3 injections at 3 weeks intervals but none of them showed any evidence of infection. Autopsies, which were performed on chloroformed animals 1 to 4 months following the last injection, were also negative.

Intracerebral inoculation of the guinea pig fetus, following the technic of Woolpert (1936), was also attempted. The head of a 30–40 day old fetus is easily palpable and was held between the thumb and forefinger for the inoculation. The abdominal skin was cleansed with alcohol; the needle was inserted through the wall and 0.1–0.2 ml. of agar emulsion was injected into the fetal brain. In no case did the female abort and all the guinea pigs were born alive. One of them did die two hours after birth. Its brain was cultured and a portion of the brain plus the liver and

lungs were ground up in a mortar and injected into another fetus but no actinomycetes could be demonstrated. Stained sections showed some perivascular cuffing but there was no other evidence of infection.

After strain D-14 had become adapted to growth in broth, it was possible to inject animals intravenously with heavy suspensions of the organism. For these animal experiments the organism was grown in stoppered large (25 mm.) culture tubes. At the end of 2–3 weeks the contents of 6–12 tubes were transferred into sterile 250 ml. centrifuge bottles, centrifuged, the broth was pipetted off, and then a dense broth suspension was prepared for the inoculations.

It was assumed that these organisms would have a very low, if any, degree of pathogenicity for laboratory animals. Thus, the dosages and inoculation schedules were instituted for the purpose of immunization rather than the production of experimental actinomycosis. However, when the animals began to lose weight and show all the symptoms of a chronic progressive disease, I began to realize that I was actually producing experimental actinomycosis in both rabbits and guinea pigs.

A total of five rabbits were injected intravenously. The first one received an initial dose of 2 ml. and two subsequent doses of 5 ml. at 3-week intervals. The animal died 16 days following the last injection. At autopsy there was noticeable emaciation; the liver was mottled; the lungs were adherent to the diaphragm, edematous, congested, and contained abscesses; the spleen contained a single nodular lesion. Microscopically the liver showed areas of focal necrosis but no actinomycetes; the lungs showed areas of consolidation, edema, and many extensive noncircumscribed abscesses, some containing granules of actinomycetes with *typical hyalinized clubs;* the spleen had an abscess with mycelial filaments.

The second rabbit received graduated doses of 1 ml., 2 ml., and 3 ml., at 3-week intervals and died three days following the last injection. At autopsy the liver showed lesions of various sizes; the lungs were edematous and congested but there were no discrete lesions; the spleen was congested; the kidneys were

mottled; there were three hard nodules along the marginal vein of one ear. Microscopically the liver showed one large and several small abscesses, the large one showed granulation tissue, giant cells, foam cells and actinomyces granules but no clubs. The lungs were congested, edematous, and had circumscribed abscesses with giant cells and actinomyces granules but no clubs. The kidneys had scattered areas of necrosis and one of these contained filaments of actinomyces. The ear contained circumscribed abscesses with actinomyces granules but no clubs.

The third rabbit received two injections and then died of snuffles.

The fourth rabbit received a first injection of 1.5 ml., a second of 3 ml., and a third injection of 5 ml. at three-week intervals and died six days following the last injection. This animal weighed 2352 grams at the time of the first injection but only 1487 grams the day before death, a loss of 865 grams in two months. At autopsy the liver was very dark and had two abscesses about 1 cm. in diameter; the lungs were congested and contained several nodular lesions. Microscopically the liver showed areas of necrosis but no actinomyces; the lungs had areas of congestion, edema, numerous non-circumscribed abscesses, some of which contained actinomyces granules *with hyalinized clubs.*

The fifth rabbit received four injections with the following amounts: 1.5 ml., 3 ml., 5 ml. and 5 ml. at three-week intervals. It was chloroformed on the fifth day following the last injection because of extreme emaciation and weakness. At the beginning it weighed 2832 grams but only 1578 grams on its last day. At autopsy the liver was pale with one large and several small abscesses; the lungs showed areas of congestion and serous exudate in the pleural cavity. Microscopically the liver had circumscribed abscesses with giant cells, foam cells and actinomyces granules but no clubs; the lungs contained several circumscribed abscesses which resembled tubercles; there were epitheliod cells, giant cells, a peripheral zone of small lymphocytes, but instead of the centers being necrotic they contained actinomyces granules *with hyaline clubs.*

One guinea pig was inoculated intraperitoneally with the broth suspensions. It received seven injections of 2 ml. each at intervals of three weeks and died six days following the last injection. At autopsy the animal was extremely emaciated; the intestines were adherent to the abdominal wall and liver; there were small abscesses in the omentum, intestinal mucosa, liver, diaphragm and lungs. Microscopically the liver showed extensive fatty degeneration and circumscribed abscesses with granulation tissue, giant cells, polymorphonuclear leucocytes and granules of actinomyces but no clubs; the lungs had areas of consolidation, congestion, edema and abscesses containing mycelial filaments; the intestines and omentum showed actinomyces granules but no clubs; the diaphragm had numerous abscesses with giant cella and actinomyces granules but no clubs.

These results show that chronic progressive actinomycosis was produced in four out of five rabbits and one guinea pig. The fifth rabbit died of snuffles. The chronic nature and progressiveness of the disease is well indicated with the fourth rabbit. It lost weight steadily.

As to the lesions themselves, there was nothing in their gross appearance which would differentiate them from any other infectious process. It is noticeable that in the rabbits, the only organ which was consistently involved was the lung. There is no apparent reason for this except that possibly the actinomycetes were concentrated in the lungs due to the extensive capillary networks. Abscesses sometimes developed along the marginal ear veins of the rabbits following the injections. In the second rabbit they developed more extensively than in the others and the actinomycetes were demonstrated in stained smears, sections, and also isolated in pure culture. The usual microscopic picture was that of a typical abscess. Polymorphonuclear leucocytes were the predominant cells, although granulation tissue, macrophages and giant cells were sometimes seen. In the second and fifth rabbits, peculiar vesicular cells were seen at the periphery of the liver lesions and because of their appearance are called "foam cells". Similar cells are found in leprosy and have been noted in human actinomycosis and in madura foot. The fifth

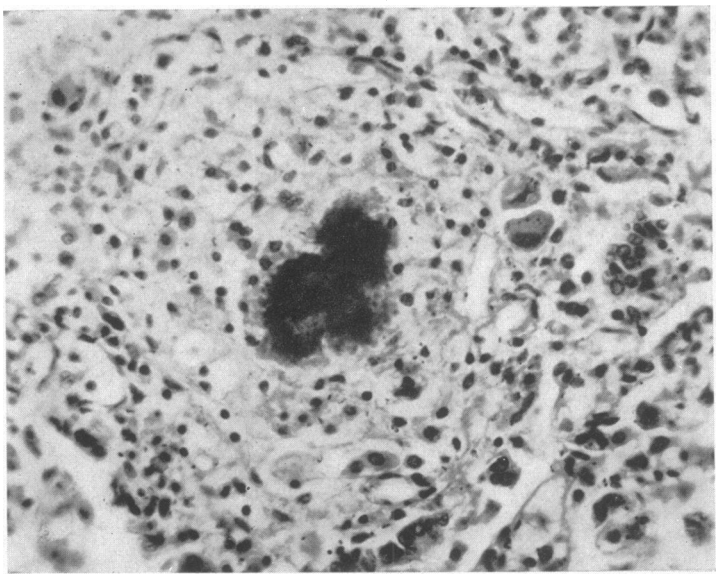

FIG. 3. TUBERCLE-LIKE LESION WITH AN ACTINOMYCES GRANULE IN THE LUNG OF A RABBIT REPEATEDLY INOCULATED INTRAVENOUSLY WITH A CULTURE FROM A PYORRHEA POCKET

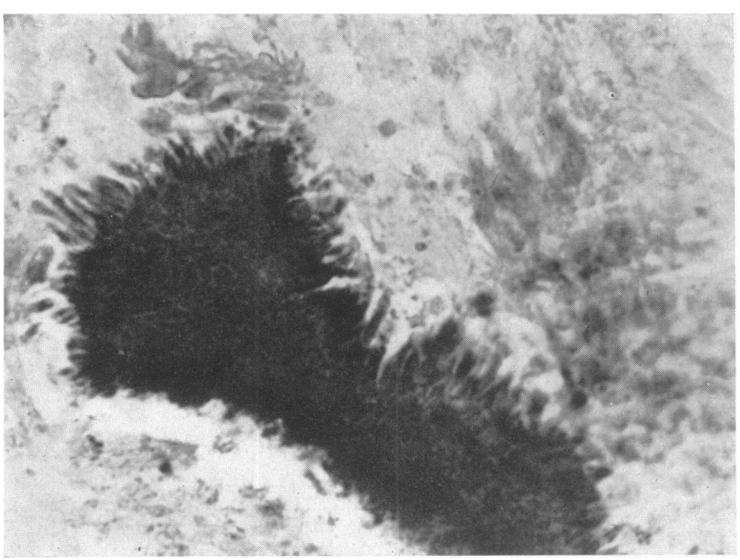

FIG. 4. ACTINOMYCES GRANULE SHOWING CLUB FORMATION IN THE LUNG OF A RABBIT INOCULATED INTRAVENOUSLY WITH A CULTURE OF AN ANAEROBIC ACTINOMYCES FROM PYORRHEA

rabbit also showed tubercle-like lesions in its lungs. These resembled tubercles in every respect except that in the center there was an actinomyces granule instead of necrotic tissue. Figures 3 and 4 are photomicrographs of actinomycotic lesions in rabbits.

Actinomyces granules with hyaline clubs (fig. 4) were found in the lungs of three rabbits. These resembled in every way the sulfur granules of human and bovine actinomycosis. There was the central mass of mycelium with the radiating peripheral hyalinized clubs. In one section stained by Gram's method, the central filaments in the clubs were clearly visible.

DISCUSSION

The exogenous theory for the source of infection in actinomycosis depends upon circumstantial evidence for support. This evidence is derived from case histories and the fact that awns of grass and grain and foreign bodies are frequently observed in these lesions.

The endogenous theory is also supported by circumstantial evidence in the form of case histories. But in addition, this theory is supported by concrete evidence from the laboratory. Anaerobic species of actinomycetes have been isolated from the normal mouth, from tonsils, from carious teeth and from pyorrhea pus. These organisms are not distinguishable morphologically from the true agents of actinomycosis and they have similar cultural characteristics.

As has been noted, the anaerobic species of actinomycetes which we isolated from tonsils and from pyorrhea pus were identical morphologically with those obtained from cases of actinomycosis. Strain D-14 also showed similar cultural characteristics and was pathogenic for laboratory animals. From these results it is possible to say that these parasitic actinomycetes are closely related to, if not identical with, the true agent of actinomycosis.

My cultural studies thus support the evidence of Naeslund and of Emmons. I have been unable to find in previous literature any reports of the production of progressive fatal actinomycosis with strains isolated from the mouth. The fact that this was accomplished in my experiments with a strain isolated from pyorrhea

pus greatly strengthens the endogenous theory, especially since the inoculated culture produced typical sulfur granules with hyaline clubs in the tissues.

Thus, in the light of these recent experiments, the most probable source of infection in actinomycosis is the oral cavity where these parasitic actinomycetes grow and become pathogenic when the proper conditions arise. What these conditions are no one knows, but from the fact that foreign bodies are commonly found in actinomycotic lesions, it is evident that trauma sometimes plays an important part. Also, certain animal experiments have indicated that sensitization is necessary to produce experimental actinomycosis, and it is possible that sensitization is important in the human disease.

SUMMARY

1. From 100 pairs of tonsils colonies of anaerobic actinomycetes were found in 14 per cent of the primary mixed cultures and pure cultures were isolated from 11 per cent of the tonsils. *Monilia albicans* was isolated from 12 per cent of the tonsils.

2. From 100 samples of pyorrhea pus, colonies of anaerobic actinomycetes were found in 18 per cent of the primary mixed cultures and pure cultures were isolated from 12 per cent of the cases. *Monilia albicans* was isolated from only one case.

3. A culture from a pyorrhea case and one from human actinomycosis were found to be identical in cultural characters.

4. Progressive, fatal experimental actinomycosis with sulfur granules was produced in four rabbits and one guinea pig inoculated with an anaerobic actinomyces isolated from pyorrhea pus.

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REFERENCES

BOSTROEM, O. 1891 Untersuchungen uber die Aktinomykose des Menschen. Beitr. path. Anat., **9**, 1–240.

DAVIS, D. J. 1914 The actinomyces-like granules in tonsils. J. Infectious Diseases, **14**, 144–158.

DAVIS, D. J., AND HALL, A. K. 1924 Studies of fusiform bacilli and spirochetes. Fusiform bacilli and spirochetes in relation to pharyngomycosis. J. Infectious Diseases, **34**, 203–208.

EMMONS, C. W. 1935 Actinomyces and actinomycosis. Puerto Rico J. Pub. Health Trop. Med., **11**, 63–76.

EMMONS, C. W. 1936 Strains of *Actinomyces bovis* isolated from tonsils. Puerto Rico J. Pub. Health Trop. Med., **11**, 720–727.

EMMONS, C. W. 1938 The isolation of *Actinomyces bovis* from tonsillar granules. U. S. Pub. Health Repts., **53**, 1967–1975.

LORD, F. T. 1910 The experimental production of actinomycosis in guinea pigs inoculated with the contents of carious teeth. Boston Med. Surg. J., **163**, 82–85.

LORD, F. T., AND TREVETT, L. D. 1936 The recovery of actinomyces-like organisms from the normal mouth. J. Infectious Diseases, **58**, 115–120.

MATHIESON, D., HARRISON, R., HAMMOND, C., AND HENRICI, A. T. 1935 Allergic reactions of actinomycetes. Am. J. Hyg., **21**, 405–421.

NAESLUND, C. 1925 Studies of actinomyces from the oral cavity. Acta Path. Microbiol. Scand., **2**, 110–140.

NAESLUND, C. 1929 Experimentelle Übertragung der Aktinomykoses von Menschen auf Versuchstiere mittels Reinkulturen der Actinomycesform Wolff-Israel (Act. Israeli). Acta Path. Microbiol. Scand., **6**, 66–77.

NAESLUND, C. 1931 Experimentelle Studien ueber die Aetiologie und Pathogenese der Aktinomykose. Acta. Path. Microbiol. Scand. Supplement 6, **8**, 1–156.

NEGRONI, P., AND BONFIGLIOLI, H. 1939 *Actinomyces israeli* (Kruse). Physis (Rev. soc. argentina ciencias naturales) **15**, 159–192.

PILOT, I., AND DAVIS, D. J. 1918 The streptococci of the actinomyces-like granules of the tonsils. J. Infectious Diseases, **23**, 562–567.

RUGE, H. 1896 Ueber Actinomces-ähnlich Gebilde in dem Tonsillen. Z. klin. Med., **30**, 529–537.

SULLIVAN, H. R., AND GOLDSWORTHY, N. E. 1940 A comparative study of aerobic strains of actinomyces from clinically normal mouths and from actinomycotic lesions. J. Path. Bact., **51**, 253–261.

TUNNICLIFF, R. 1926 The organism of actinomyces-like tonsillar granules. J. Infectious Diseases, **38**, 366–370.

TUNNICLIFF, R., AND JACKSON, S. 1930 *Vibriothrix tonsillaris* N. Sp. The organism of actinomyces-like tonsillar granules. J. Infectious Diseases, **46**, 12–17.

WILKINSON, H. F. 1928 A study of the pathology of 10,000 pairs of tonsils. Thesis, University of Minnesota, Minneapolis.

WOLFF, M., AND ISRAEL, J. 1891 Ueber Reincultüre des Actinomyces und seine Uebertragheit auf Theire. Archiv. path. Anat. (Virchow's) **126**, 11–59.

WOOLPERT, O. C. 1936 Direct bacteriological experimentation on the living mammalian fetus. Am. J. Path., **12**, 141–151.

WRIGHT, JAMES 1905 The biology of the microorganisms of actinomycosis. J. Med. Research, **13**, 349–398.

WRIGHT, JONATHAN 1904 Actinomycosis of the tonsils. Am. J. Med. Sci., **128**, 74–90.