ACTINOMYCOSIS

Report of a Case

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Actinomycosis, caused by *Actinomyces bovis,* is a chronic granulomatous suppurative disease characterized by intensive induration and dark red discoloration, followed by development of deep abscesses, which eventually rupture and leave persistent multiple draining sinuses, and the appearance of tangled mycelial masses (granules) in the discharges and in tissue sections.

Actinomycosis has been reported in nearly all parts of the globe. According to Zachary Cope, the fungus has been found to be the cause of disease wherever there is a microscope and a laboratory and that the more carefully the fungus is sought, the more often it is found.

Actinomycosis affects man, cattle and other animals. According to the history of this infection given by Lewis and his associates (4), the disease called "lumpy jaw" in cattle was first described by Bollinger in 1877. Harz first described *Actinomyces bovis* from pathological materials obtained from a case of "lumpy jaw" in cattle and named the disease actinomycosis. He characterized the etiologic agent not by culture but by its appearance in materials from the tissues. The tiny masses of fungi in pus and tissues led him to name the organism Actinomyces (the ray fungus). The disease was first recognized in humans by Israeli, and Ponfick shortly after pointed out the similarity of the disease described by Bollin-

ger in cattle to the infection which Israel had observed in man. The etiologic agent was finally obtained in pure culture by Wolf and Israel in 1891 from human actinomycosis and proved its pathogenicity by inoculations of animals with pure cultures. Rosebury (8) cited several workers who subsequently isolated this organism from man, cattle, and other animals.

The organism isolated from human cases was called *A. bovis* with the modifying phrase *"Wolf-Israel type"*. Rosebury (8) cited several workers who later used the specific *Actinomyces israeli* to designate the anaerobic or microaerophilic parasitic actinomycetes which were isolated from human cases. It was, however, shown later that the organisms from bovine and human lesions were identical in appearance and that both were similar in culture, requiring an anaerobic or microaerophilic condition for growth. According to Smith and Conant (13) since the Wolf-Israel actinomycete could be cultured only by microaerophilic methods and repeated culturing by by other investigators showed this to be true for the organism of both human and bovine actinomycosis, the microaerophilic *A. bovis* has become established as the etiologic agent of the disease.

The possibility that different species of anaerobic actinomycetes caused actinomycosis in man and animals had been considered by some workers (2, 14). Results of these studies tend to show differences between the bovine and human isolates in colony formation (rough and smooth), oxygen tolerance, fermentation, and antigenic response. Strains of human origin produce rough type colonies, and were called by Erikson (2) *A. israeli*, while strains of bovine origin produce smooth type colony and were called *A. bovis*. The human strains were more active on sugars than the bovine, produced mycelia in colonies, which bovine strains did not do and were antigenic, while bovine strains were not. Thompson (14) in 1950 corroborated the findings of Erikson on the existence of two distinct species of pathogenic anaerobic Actinomycetes. He, like Erikson, was for the use of the specific name *A. israeli* for the rough human strains and *A. bovis* for the smooth bovine strain. Thompson, however, isolated one strain of *A. israeli* from bovine sources and pointed out that, possibly, some bovine infections maybe caused by this species. He,

likewise, cited the work of Holm and Luetze who isolated 6 out of 69 strains with smooth colonies which produce diffuse growth in broth, from typical human cases, as an indication that probably a small number of human infection may be due to *A. bovis*. The sixth edition of Bergy's Manual of Determinative Bacteriology lists two species of Actinomyces: *A. bovis* from human sources (mouth, tonsillar crysts, etc.) and *A. bovis* from cattle and probably other animals. Rosebury (10) believes that a distinction between the smooth *A. bovis* isolated from cattle and the rough *A. israeli* from man may not be tenable because of the fact that both smooth and rough forms may be found in either host species, and the rough strains may become smooth after cultivation. Furthermore, agglutination reaction done by Slack and associates (12) failed to demonstrate antigenic difference among 20 strains of microaerophilic actinomycetes from human and various animal sources.

A. bovis belongs to a large group of organisms belonging to the Order Actinomycetales of the Class Schizomycetes. The actinomycetes, as they are called, stand midway between the true bacteria and the molds in their properties. In size, they are like the true bacteria, being 1 micron or less in width. Some reproduce like the bacteria, as shown by the members of genus actinomyces which fragment readily into bacillary or coccoid form. Like many bacteria, they are sensitive to penicillin and other antibiotics and sulfa drugs. The actinomycetes on the other hand, are related to the higher filamentous mold in that they exhibit true branching. The classification of these actinomycetes by Waksman and Henrici (17) which has been generally accepted is given in Table I.

Table 1. Classification of the Actinomycetales (Waksman and Henrici, 1943) (17)

I. Mycelium rudimentary or absent	Family Mycobacteriaceae Chester.
A. Acid-fast organisms	Mycobacterium Lehman & Neumann.
II. True mycelium produced.	
A. Vegetative mycelium fragments into bacillary or coccoid elements.	Family Actinomycetaceae Buchanan
a. Anaerobic or microaerophilic, parasitic, not acid-fast.	Actinomyces Harz.
b. Aerobic, partially acid-fast or nonacid-fast.	Nocardia Trevisan

B. Vegetative mycelium not fragmenting into bacillary or coccoid elements.	Family **Streptomycetaceae** Waksman and Henrici.
a. Multiplication by conidia in chains from aerial hyphae.	**Streptomyces** Waksman and Henrici.
b. Multiplication by single terminal spores on short sporophore.	Micromonospora **Orskov.**

The theory of Bostroem that actinomycosis is an exogenous infection derived by traumatization with grass, straw or grain which carries the infecting agent had been entertained for sometime. Present knowledge, however, favors the endogenous theory of infection. Smith and Conant (1) cited numerous investigators including Lord (1910), Naeslund (1925), Emmons (1938), Sullivan (1940), Slack (1942), Rosebury (1944), and Thompson (1950) who have shown that the anaerobic actinomycetes have been found to be a normal inhabitant of the mucous membrane of the mouth and has been isolated from the tartar of the teeth, detritus around carious teeth, from pyorrhea pus, and from materials from tonsillar crypts, but has not been isolated from natural substrates. Rosebury (9) and Slack (11) found that strains isolated from so-called normal mouth were similar to or indistinguishable from the strains derived from cases of actinomycosis. Furthermore, experimental actinomycosis has been produced with strains (anaerobic or microaerophilic actinomycetes) isolated from the indigenous flora of the mucous membrane in man (5, 6, 9, 11). There has been no report of man-to-man or animal to-animal transmission. From these observations, the most probable source of infection of actinomycosis is the oral cavity where these parasitic actinomycetes grow and become pathogenic when the proper conditions arise. It is not known what these proper conditions are. Trauma, sensitization to the organism and the role of other bacteria have been considered as probable essential factors in the pathogenicity of actinomycosis (11).

A. bovis has only slight and irregular pathogenicity for laboratory animals. Early workers trying to demonstrate pathogenicity of *A. bovis* on common laboratory animals were usually unsuccessful or obtained only localized lesions. Re-

peated passage, trauma during inoculation or admixture with other bacteria, or foreign body have not produced regular progressive infection, and were generally ineffectual. Repeated inoculations at intervals that might sensitize the animal, have resulted in some instances (though not regularly) in progressive fatal experimental actinomycosis, indicating that allergic sensitization may be a factor in the pathogenesis of actinomycosis. Meyer and Verges (6) reported in 1950 that progressive infection was obtained in almost 95% of 93 young albino mice injected intraperitoneally with a suspension of pure culture of *A. bovis* (from human source) mixed with gastric mucin. Hezen and associates (3) reported that the hamster is a suitable animal in demonstrating the pathogenicity of *A. bovis*.

The clinical picture of actinomycosis varies with the location of the disease. The disease is classified into cervicofacial, thoracic, and abdominal actinomycossis depending on the site of the initial infection. The most common form of infection is the cervicofacial, which is believed to be the result of the entrance of the organism through the mucous membrane of the mouth and pharynx, by way of the gums about carious teeth, through the tonsils or by direct extension through the ducts which may result in infection of salivary glands. Conant and his associates (1) describe cervicofacial actinomycosis as follows: "Most frequently, the infection is noted first in the lower jaw, particularly in the region of an infected tooth or in the socket left by a recent extraction. A history of previous toothache or other dental affection frequently is obtained. The swelling usually is most marked over the angle of the mandible, but may be posterior to it if the fungus gained entrance through the tonsils.

The swelling in the soft tissues of the face is not characteristic at first; but the overlying skin soon assumes a dark red or purplish color, the tumor develops a "wooden" type of hardness and the surface appears uneven or "lumpy". As the disease progresses, abscesses develop and multiple sinuses appear. Trismus is a frequent symptom when the muscles of mastication are affected. Pain is minimal unless there is a marked secondary infection, and the general health of the pa-

tient remains good if the disease is localized in the face and neck".

The organism in the mouth may be aspirated into the lungs to cause the thoracic form, developing a condition simulating pulmonary tuberculosis.

The organism in the mouth may be swallowed and produce the abdominal form, characterized by the development of irregular masses along the colon, but most frequently in the ileocecal region producing a condition simulating appendicial abscess.

CASE REPORT

R. L., 27 years, male, Filipino, employee of an electrical lamp manufacturing firm, and residing in Las Piñas, Rizal, first consulted at the company clinic on August 17, 1961 for severe pain in the left lower first molar and slight fever.

The patient was apparently free from venereal disease. His mother and a brother died of pulmonary tuberculosis, the latter less than a year ago. The other members of the family, including his father and sisters are well.

On June 16, 1961, while doing quality control test, a 1000-watt bulb suddenly exploded, wounding him on the face, neck, cheek, and elbow mostly of the right side (opposite that of the present lesion). Pieces of broken glass had to be removed from the wounds. (The bulbs tested contain argon, phosphorous, and nitrogen).

The patient was first seen by one of us (E.L.) on August 17, 1961. He had slight fever and was complaining of pain in the left lower first molar. He was given analgesic tablets and referred to the company dentist. An abscess of the gum which developed at the region of the left lower first molar was incised, but the pain, instead of subsiding, became more severe and persistent. Analgesic tablets given by the company nurse enabled him to continue working. He was later referred to a dental school infirmary where X-ray taken of the left jaw showed an infection of the root of the painful tooth. This tooth was extracted under local anesthesia but no relief from pain was felt.

When the removal of the affected tooth failed to relieve the pain, he was given antibiotics and a dental prophylaxis. This did not relieve him of the pain. Another dentist he consulted when the pain spread to the other teeth of the same side suspected pyorrhea and gave him Terramycin. All these and the penicillin tablets which the patient took on his own, were of no benefit. Finally, the dentist extracted all the lower incisors and gave him Penbid tablets. He developed a severe allergic reaction to the penicillin which was relieved by Benadryl capsules and Decadron tablets.

After the extraction of the incisors, the patient noticed that the pain transferred to the angle of the left lower jaw and there was gradual swelling of the same area. The affected part was very hard but not tender nor red. He also developed slight difficulty in opening his mouth. This time he was given triple sulfa tablets but the swelling increased.

On November 1, 1961, he saw another physician who gave him a skin test for penicillin sensitivity. He developed a severe general reaction which was relieved by Decadron tablets. Due to the sensitivity to penicillin, the physician gave him Iodex locally and capsules of Chloromycetin which he took for three days without any beneficial effect.

He was referred to the Philippine General Hospital where he was given Chloromycetin capsules and Icthyol-belladonna ointment. After a week, the swelling at the angle of the jaw softened and incision and drainage was done. The periphery of the lesion remained hard inspite of the incision and intake of Ledercylin tablets; and the swelling gradually spread to the left side of the neck. The affected part of the neck was at first hard and tender but later there was softening and a reddish discoloration. The X-ray of the face taken on December 5, 1961 showed osteomyelitis of the jaw involving the region of the molars and extending up to the angle of the jaw. X-ray of the lungs taken on December 19, 1961 showed essentially clear lung fields. In the mean time, the swelling spread to the lower portion of the chest. The patient became febrile, and was unable to move his neck freely. On December 14, 1961, incision and drainage of the neck lesion was done at the National Orthopedic Hospital. The discharge, mostly pus and

blood was sent to the Institute of Hygiene for bacterial culture and sensitivity test. About three weeks later, the patient returned to the Institute of Hygiene with a fluctuating swelling just above the angle of the jaw (Fig. 1). This abscess was aspirated and the pus examined mycologically.

MYCOLOGICAL EXAMINATION

The clinical material examined consisted of blood tinged pus obtained by aspiration of a fluctuating mass in the left side of the neck.

The pus, which was collected in a sterile test tube, when allowed to spread on the sides of the tube showed numerous granules (Fig. 2). The pus was transferred to a Petri dish and the granules removed by means of sterile capillary pipette and washed several times with normal salt solution.

The granules were irregularly spherical varying in sizes from a fraction of a millimeter to about 2 mm. in diameter. They were soft and easily pressed flat on a slide by gentle pressure on a coverslip. The color varied from hyaline to light or pale yellow (sulfur yellow). The small ones were more or less transluscent and hyaline while the larger ones were yellowish.

The granules were irregularly spherical varying in size stained slide-covership preparation and in Gram-stained preparation of the smeared and fixed material.

In the wet unstained preparation, the granules have a characteristic radiating lobulated structure when seen with the low power lens (Fig. 3). With higher magnification the interior of the granule was shown to be made up of a mass of small filaments hardly distinguishable (Fig. 4), but in many instances, swollen structures or clubs were seen radially arranged at the borders of the lobules (Fig. 5).

The granules when crushed between two glass slides, spread in a thin film, fixed, and stained by Gram's method were seen as Gram positive branching filaments or diphtheroid hyphal elements (Fig. 6). Clubs which were seen in wet preparation were no longer present. The individual filament measured 1 micron or less in width.

Isolation of the organism was done by inoculating several kinds of culture media. Since the specimen was obtained from a closed lesion, bacterial contamination was not expected to cause difficulty in the isolation of the actinomycete.

The washed granules were crushed and inoculated into (1) Brewer's thioglycollate broth, (2) brain heart infusion broth (the broth fills more than one half of the test tube), (3) brain heart infusion agar shake tube and, (4) brain heart infusion agar plate. The first 3 media were incubated at 37° C. under aerobic condition. The brain heart infusion agar plate was inoculated by streaking the material on the surface and was provided with an anaerobic environment using a modification of the method used by Mossel for isolation and study of obligate anaerobes (7). The plate was also incubated at 37 C.

In thioglycollate broth, the organism grew in about five days as small fluffy white colonies throughout the medium beginning about ½ cm. from the surface. On retransfer of the organism from the original tube of thiolycollate broth to the same medium, the growth was seen as white, crumb-like colonies of varying size throughout the medium except in the upper 1½ cm. (Fig. 7).

In the tall column of brain heart infusion broth, the organism grew in the bottom of the test tube as an irregular, white colony with lobulated edge easily broken up by shaking the tube (Fig. 8). The broth remained clear.

In brain heart infusion agar shake tubes, the organism grew as white, lobulated, compact colonies in the depths of the medium beginning about 1½ cm. from the surface. The colonies reached a size of about 2-3 mm. in one week (Fig. 9). The surface colonies of the organism on brain heart infusion agar incubated anaerobically at 37 C. for one week, were of the rough type (Fig. 10). They were usually about 1 mm. in diameter but colonies as large as 3 mm. were occasionally seen especially when widely separated from one another. The colonies were white, opaque, raised or "heaped-up" and irregular in both surface and outline. Magnified with a hand lens, the colonies showed a glistening though irregular surface. The colonies were strongly adherent to the medium, so that it was hard to remove them with the inoculating needle.

Such colonies were hard to emulsify when smears were made from them.

Smears prepared from the growth in brain heart infusion broth and thioglycollate broth stained with Gram's method, showed the organism as short twig-like and branching forms which are Gram-positive (Fig. 11). The organism was not acid-fast. Smears prepared from the organism growing on solid medium showed the organism mostly as Gram-positive diphtheroid hyphal elements. These diphtheroid forms resulted from the fragmentation of the filaments which occurred during the preparation of the smear.

Based on the morphological characteristics, staining reaction, and cultural characteristics, the organism that was isolated from the patient was *A. bovis.*

ANIMAL PATHOGENICITY TEST

Meyer and Verges (6) have developed a reliable mouse pathogenicity test as a diagnostic aid in the identification of *A. bovis.* This method was used to test the pathogenicity of the isolated organism.

The organism was grown on brain heart infusion glucose agar plate incubated anaerobically at 37 C. for 5 days. The colonies were loosened by scraping the surface of the plate with a hard inoculating needle and washed off with normal salt solution. The suspension was adjusted to match tube 4 of the McFarland nephalometer. One part of the suspension was mixed with one part of 5% mucin * and 0.5 ml. of the resulting mixture injected intraperitoneally into each of 5 young male albino mice weighing 10-15 grams.

One mouse died 48 hours after inoculation but on autopsy no gross lesions were observed. Another mouse died 7 days after inoculation and a third, 10 days after. Autopsy of these two mice showed lesions on the liver and stomach. The lesions consisted of abscesses appearing as whitish or cream-colored nodules varying in size from 1 to 4 mm. in diameter. The inferior surface of the liver was most affected (Fig. 12).

* Granular mucin, Type 1701-W, from the Wilson Laboratories, Chicago, was used.

Two mice were sacrificed 30 days after inoculation. One showed an abscess about 1 and ⅔ mm. in diameter at the margin of the left lobe of the liver and a similar smaller abscess on the serosa of the stomach. In the other mouse, there were no lesions in the liver or stomach, but two abscesses were seen in the fat of the omentum.

An abscess when crushed on a slide produced a thick creamy material.

Material from the abscess was cultured by streaking on the surface of brain heart infusion glucose agar plate provided with anaerobic condition and incubated at 37 C. Rough colonies of *A. bovis* like those obtained in the primary culture from the patient were obtained. Gram-positive filaments of varying lengths were seen in smears from the colonies.

Creamy material from an abscess, when smeared and stained by Gram's method, showed numerous Gram-positive filaments of varying length, some with branching. Histological section from the lesion (abscess in the omentum) stained with hematoxylin and eosin showed an abscess with the characteristic lobulated granules (Fig. 13, 14). The granule was surrounded with abundant lymphocytes and polymorphonuclear leukocytes. The wall of the abscess was made up of adipose tissue infiltrated with some polymorphonuclear leukocytes, lymphocytes and occasional macrophages. The central portion of the granule stained more heavily with hematoxylin. The individual filaments comprising the granule could not be clearly defined, but those found in the periphery tended to assume a radial arrangement and in some areas there was deep staining with eosin. Sections stained by Gram's method showed the granule to be made up of Gram-positive branching filaments.

IN-VITRO SENSITIVITY TO PENICILLIN AND TERRAMYCIN

The isolated actinomycete was grown on thioglycollate broth for 5 days. The broth was centrifuged and the sedimented organism was suspended in normal salt solution to

make a sufficiently thick suspension. This was used as inoculum in the in-vitro sensitivity test.

A solution of the antibiotic was added to melted, cooled brain heart infusion glucose agar and allowed to solidify in a slanted position. The following concentrations of penicillin (in units per ml. of medium) were employed: 0, 0.2, 1, 5, 25, and 125. For Terramycin, the concentrations employed (mcg. per ml. of medium) were: 0, 1, 5, 25 and 125.

The antibiotic agar was inoculated with a loopful of the suspension of the test organism. All tubes were provided with anaerobic condition by absorbing the oxygen (in sealed tube) by means of alkaline pyrogallic acid. Incubation was done at 37 C. and the tubes were examined at suitable intervals for the presence of growth. Results of the test are shown in Table 2.

Table 2

In-vitro Sensitivity of *A. bovis* to Penicillin and Terramycin

PENICILLIN Concentration units/ml. of medium	5 days	10 days	15 days
0	++	+++	+++
0.2	–	–	–
1.0	–	–	–
5.0	–	–	–
25.0	–	–	–
125.0	–	–	–
TERRAMYCIN mcg/ml. of medium			
0	++	+++	+++
1	–	–	–
5	–	–	–
25	–	–	–
125	–	–	–

– no growth
++ growth
+++ luxuriant growth

It can be seen from Table 2, that this strain of *A. bovis* is very sensitive to both Penicillin and Terramycin; 0.2 units/ml. of penicillin and 1 mcg/ml. of terramycin completely inhibted the growth of the organism for 15 days.

These findings indicate the usefulness of these drugs in the treatment of the case. Concentrations of penicillin in the serum ranging from 2 to 7 units/ml. and of terramycin ranging from 3 to 6 mcg/ml. — which are much higher than the observed in-vitro inhibiting concentrations — may easily be attained by the usual method of administration of these antibiotics.

COMMENT

As far as can be ascertained, this is the first case of actinomycosis reported in the Philippines in which the causative organism has been isolated. Ther are two earlier reports on this infection in our literature. The first is a case of actinomycosis of the human jaw reported by Villa (15) in 1948. The diagnosis was based on the observation of structures morphologically that of the ray fungus in haematoxylin and eosin stained sections of decalcified tooth with attached granulomatous tissues. The second is an actinomycotic lesion in the pulp reported by Villa (16) in 1957. Diagnosis was based on the morphological appearance of the organism in haematoxylin and eosin stained section of the tooth.

While actinomycosis has been reported in other countries as common in the jaw of animals, we have been unable to find any report in the local literature. Villa (15), however, mentioned that Gomez knew of one report of a case of lumpy jaw in a Batangas bull in 1931.

It is fortunate that in this case the causative organism has been isolated and shown to be capable of causing actinomycotic lesions in young mice. These laboratory procedures furnish the most convincing evidence of infection in this patient. Although the observation of granules in pus or in tissue sections stained with haematoxylin and eosin is very suggestive of actinomycosis (and in some reported cases this is the only basis for diagnosis) it must be pointed out that there are other infections producing similar granules. Certain bacterial infections associated with foreign bodies, to which the names "botryomycosis" and "staphylococcic actinophytosis" have been given, show granules simulating those of actinomycosis. Granules are also observed in other fungus infections, parti-

cularly in mycetoma caused by *Nocardia* species and in madu-romycosis caused by the molds including *Monosporium apios-permum* and *Madurella grisea.* The observation of granules in a fresh mount or in haematoxylin and eosin section is generally not sufficient to establish the diagnosis of actinomycosis. Applying the Gram's method of staining to a smear of the granule or to the tissue section is often helpful in determining the etiologic agent. But the final identification rests on cultural studies of the organism.

It is universally accepted that pathogenic strains of *A. bovis* are found in the normal mouth and that actinomycosis is an endogenous infection of man and animals.

The patient gave a history of trauma to the face and neck caused by an explosion of an electric light bulb. Whether or not this injury has any relation to the initiation of the infection is highly speculative. It may be assumed that the organisms in the mouth were able to pass through the space between the gingival margin and the surface of the tooth, initiating an infection which later on developed into an abscess near the roots. The X-ray picture of the affected area at this stage showed evidence of infection of the roots. As a result of injury to the suroundng tissues caused by the incision of the abscess and extraction of the tooth, the organisms were able to spread to the deeper tissues thus extending the infection to the bone of the lower jaw and to the soft tissues of the face near the angle of the jaw. Evidence of the spread of the infection to the surrounding bony structure was furnished by another X-ray (December 5, 1961) which showed rarefaction and resorption of the bone in the region around the molars and extending up to the angle of the jaw.

Infection of the teeth and their supporting structures by actinomycetes are not uncommon. Villa (15), in his report of actinomycosis of the human jaw, mentions his study of some cases of disease of the supporting structures of the teeth where he observed in the majority of cases the presence of the ray fungus. In his report of pulp abscess associated with actinomyces, Villa (16), also comments on the frequent observation of actinomyces-like bodies in pulps undergoing in-

flammatory changes or necrosis particularly in pulps exposed to the outside as a result of caries.

As soon as the diagnosis of actinomycosis was made, the patient was given terramycin capsules and Midikel tablets for a period of two months. The response of the patient to the treatment was good. The pain, swelling, induration, and trismus gradually disappeared. After about 1 and ½ months of treatment, the affected area was almost normal in appearance except for some slight reddish discoloration and the slightly prominent incision scars.

SUMMARY

1. The clinical manifestations of a case of cervicofacial actinomycosis were presented.
2. Mycological methods used in diagnosis were described.
3. The morphological characteristics and staining reactions, cultural characteristics, and animal pathogenicity test identified the organism isolated as *Actinomyces bovis.*
4. The strain of *A. bovis* isolated was found susceptible to concentrations of penicillin and terramycin that are easily reached in the blood serum.

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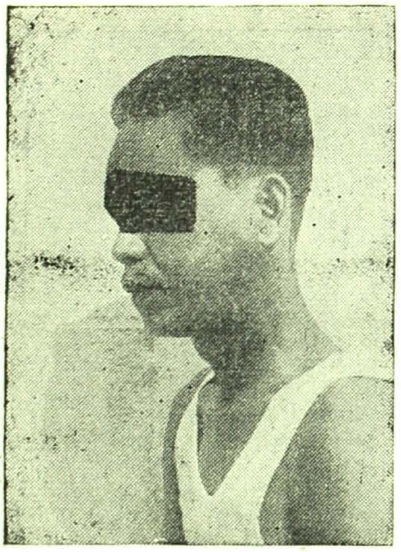

Fig. 1

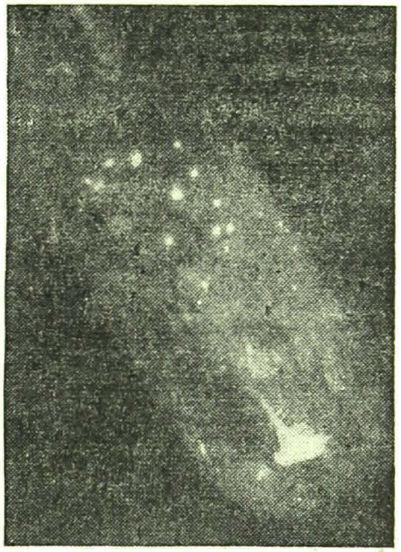

Fig. 2

Fig. 1 — Patient with a fluctuating swelling just above the angle of the jaw.

Fig. 2 — Numerous granules of pus on the sides of the tube obtained by aspirations of a fluctuating mass in the left side of patient's neck.

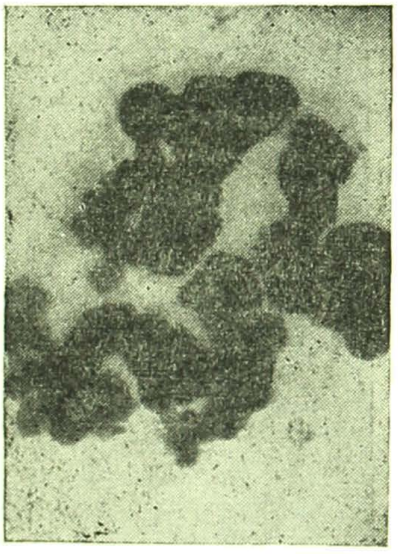

Fig. 3

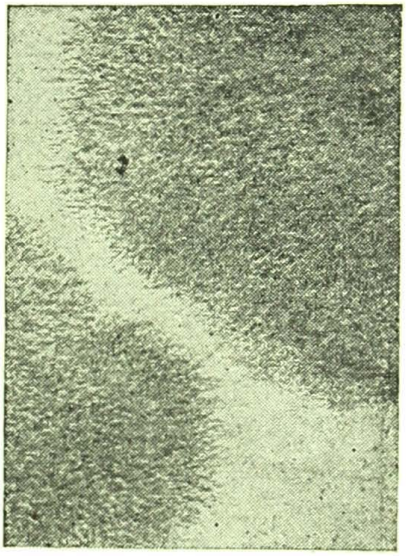

Fig. 4

Fig. 3 — Granules with characteristic radiating lobulated structure as seen in a low power lens.

Fig. 4 — Mass of small filaments hardly distinguishable as seen under a higher magnification.

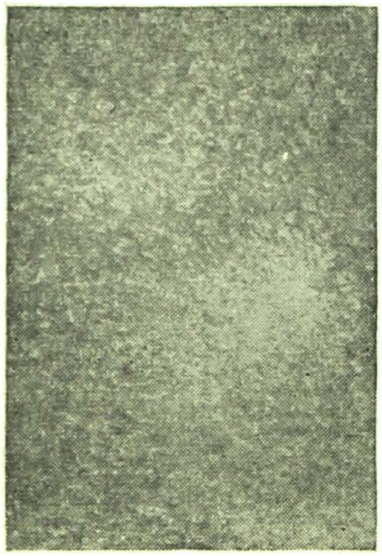

Fig. 5

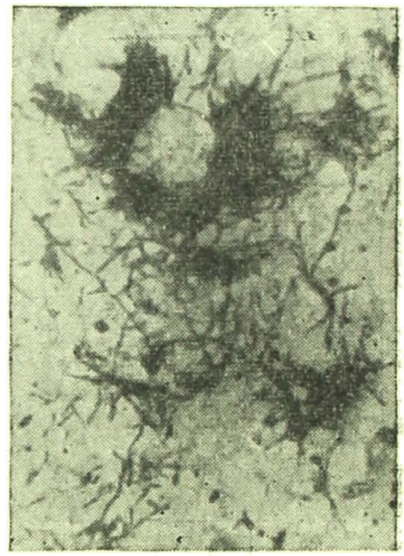

Fig. 6

Fig. 5 — Swollen structures or clubs radially arranged at the borders of the lobules.

Fig. 6 — Stained by Gram's method, granules are seen above as positive branching filaments.

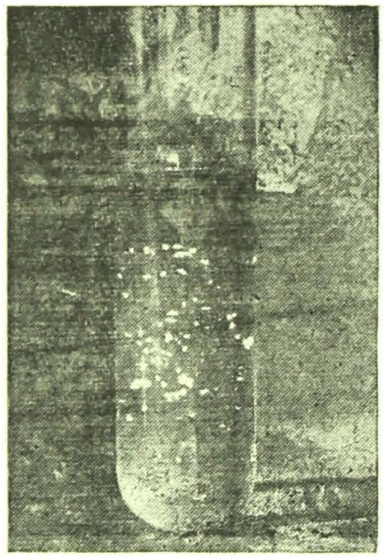

Fig. 7

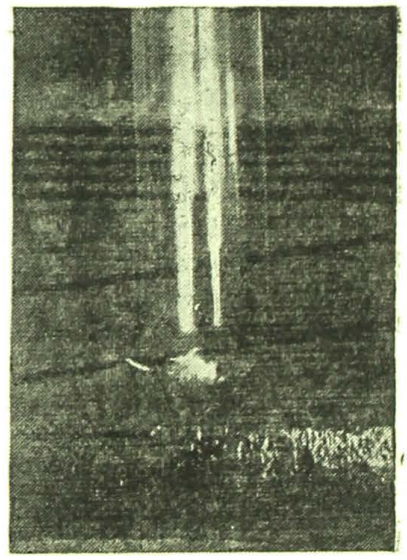

Fig. 8

Fig. 7 — White crumb-like colonies of varying sizes shown growing about 1/2 cm. from the surface.

Fig. 8 — Brain heart infusion both containing irregular white colony with lobulated edge.

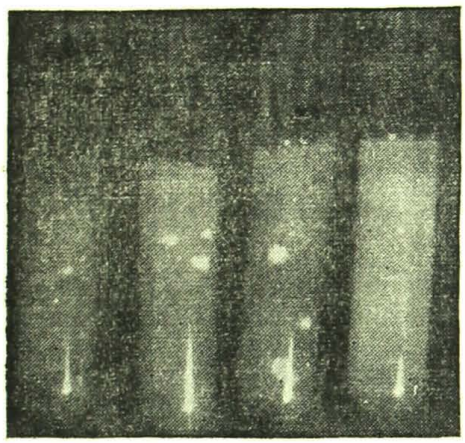

Fig. 9

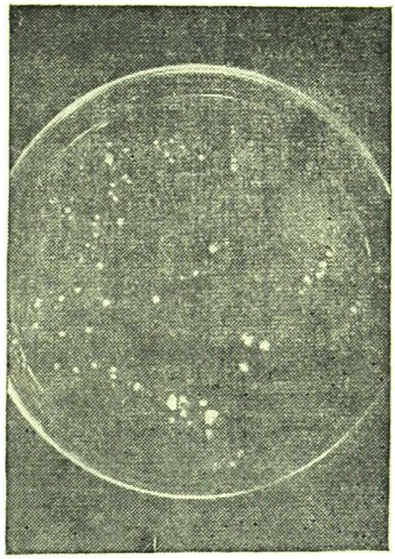

Fig. 10

Fig. 9 — Compact colonies about 2-3 mm. in one week.

Fig. 10 — Week-old surface of organism on brain-heart infusion agar incubated anaerobically at 37 C.

Fig. 11

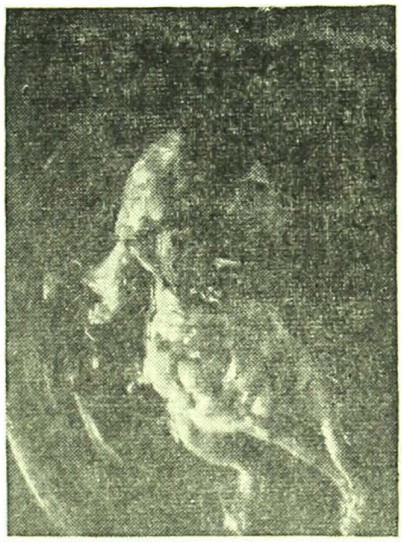

Fig. 12

Fig. 11 — Short twig-like and branching form organisms (Gram-positive).

Fig. 12 — Interior surface of mouse liver showing lesions with whitish or cream-colored nodules.

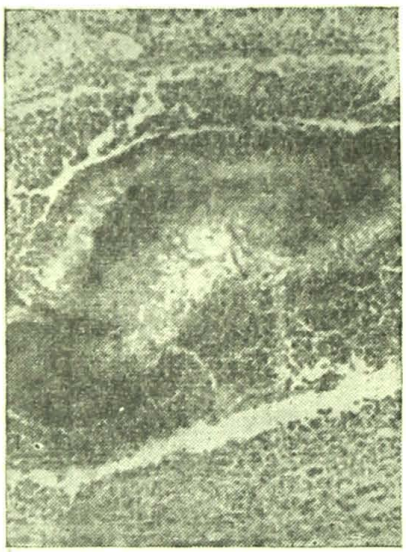

Fig. 13

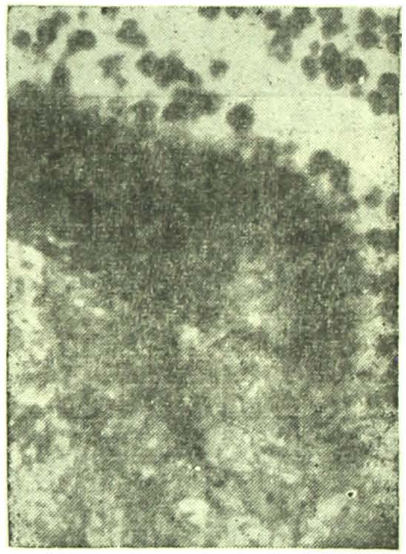

Fig. 14

Figs. 13 and 14 — Section from the liver lesions stained with hematoxylin and eosin showing abscess with characteristic lobulated granules.