TWO CASES OF NECROTIC BRONCHO-PNEUMONIA WITH STREPTOTHRIX.

By CHARLES NORRIS, M. D., and JOHN H. LARKIN, M. D.

(*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*)

Plates XI–XVI.

The rarity and importance of such cases as form the basis of this paper, the inadequacy of the study of the similar cases already recorded, and the interesting, though as yet obscure, relationship between an organism or group of organisms which have long been known as actinomyces and the more recently-named streptothricaceæ, have urged us to the somewhat extended study of our material, which it is the purpose of this paper to record.[1]

While the clinical histories are incomplete, the lesions, especially of the lungs, are well defined, and the characters and relationships of the microorganisms present seem to us to be of considerable general as well as special and technical interest.

Case I. *Clinical History.*—J. L., aged 45, carpenter, was admitted to St. Francis Hospital, December 15, 1898. No history of previous illness. For the past six weeks patient had suffered from cough and moderate dyspnœa. Breath was extremely fetid. Patient was fairly well nourished.

Physical examination on admission revealed a nearly complete consolidation of the right lung, most marked in the lower lobe. Some consolidation of the left lower lobe. The temperature was always high, ranging from 103 to 104° F. The patient did badly and, on account of his continuous coughing and the foul odor of his breath and expectoration, he was isolated.

The clinical diagnosis was gangrene of the lung, following lobar pneumonia. He grew rapidly worse and died eight days after admission.

[1] A brief preliminary report of these cases was made to the New York Pathological Society on March 8, 1899.

Autopsy (Dr. Larkin), two hours after death, December 23, 1898. Body well developed and muscular; no rigor mortis or post-mortem lividity. The abdominal cavity contained a slight amount of straw-colored serum. The left lung was bound down by old adhesions; the lymph nodes at root of lung were very large, measuring from two and a half to three inches in length and one inch in breadth. The mucous membrane of the trachea and bronchi was œdematous, intensely congested, and bestrewn here and there with whitish masses resembling actinomyces "granules." The right lung was bound down by old adhesions and entirely consolidated. Bronchiectatic dilatations were present throughout the lungs. The cut section of the lungs, which were markedly anthracotic, was smooth, glistening, and mostly dark bottle-green in color. The consolidation was firm and resembled that of an organizing pneumonia. The connective tissue septa of the lung were œdematous and grayish in color. Myriads of small yellowish-white foci were scattered through the lungs. The odor of the lungs was extremely fetid.

The kidneys, liver, spleen, pancreas, intestine, bladder, and prostate appeared normal.

Histological Examination.—The microscopic study embraced only the lungs, which were preserved in alcohol. The sections of the various lobes exhibit striking similarity in their microscopic appearances. The anthracosis is not so extensive as we were led to expect from the color and gross appearances of the lungs. The pigment is present in moderate amount in the peribronchial and pleural tissues.

Bronchi.—In places there is a simple catarrhal bronchitis with desquamation of the epithelium, fibrin and leukocytes. The coats of the bronchi are congested and infiltrated with serum and pus cells. The surrounding alveoli are filled with pus cells and a little fibrin. The simple catarrhal bronchitis and peribronchitis are confined to a few tubes. Besides the filling up of the terminal bronchioles and alveoli with leukocytes, a few small miliary abscesses or groups of alveoli filled with leukocytes are found. In the exudate of such alveoli and bronchi only a few streptococci and filaments or rods are present.

Many of the bronchi contain the filamentous colonies already mentioned as suggesting actinomyces granules at the autopsy. Where these are present the bronchi are the seat of a severe and destructive lesion, a necrotic suppurative bronchitis with dilatations, and here the bronchial structure is represented by a tissue staining deeply with eosin, composed of cells with ill-defined outlines and with fragmented nuclei. The

intimate relation of the bacterial or filamentous colonies to the necrosis is clearly seen in the places where the necrosis is strictly limited to the site of attachment of the colonies, although usually the whole circumference of the bronchus is implicated (Plate XVI). The necrosis may affect only the mucosa, or extend through the bronchial coats and about the bronchial dilatations, which contain the largest bacterial colonies, also to the adjacent, compressed and distorted zone of alveoli, which are filled with a coarse network of fibrin staining deeply with eosin. The leukocytes in the lumen of the bronchi near these colonies are in all stages of necrosis and degeneration, and the large amount of detritus found in the tubes is composed mainly of broken-down pus cells.

Pulmonary parenchyma.—The broncho-pneumonic lesion presents two phases of inflammation, which vary in importance and extent in different places. There are, first, zones of peribronchial alveoli filled with leukocytes in a marked condition of nuclear fragmentation, and with fibrin and epithelial cells. The alveolar epithelium in such regions shows little tendency to proliferation, and the alveolar walls are thickened by capillary congestion, by a cellular exudate composed of leukocytes and by epithelioid cells or fibroblasts. Adjacent to or surrounding such alveoli, are second areas of inter- and intra-alveolar pneumonia. Here the alveolar walls are much thickened by a proliferation of polyhedral or epithelioid cells and the fibrin in the alveoli is in all stages of organization. Vascularized plugs of connective tissue obliterate the alveoli here and there. In some sections the areas of interstitial pneumonia are quite extensive. These areas may directly touch upon bronchi which are the seat of an intense necrotic inflammation. Nuclear fragmentation and slight necrosis of the new tissue are observed even at some distance from the bronchus.

The adventitial coats of the arteries, and the connective tissue surrounding the larger bronchi and vessels are much thickened by a serous exudate. In places the necrosis has extended to the walls of the blood-vessels, and the alveoli are filled with red corpuscles. In some sections the hæmorrhagic areas are quite extensive.

Besides the extreme congestion of the capillaries and blood-vessels, few vascular changes exist. Some of the smaller arteries present an obliterating endarteritis. In the larger vessels considerable fibrin and a homogeneous material resembling coral-thrombi are seen. Cocci and filaments are not found in the blood-vessels. The lymph spaces of the bronchi and interlobular septa contain a few streptococci.

The pleura is covered in places by a layer of fibrin and leukocytes,

and is congested, thickened and infiltrated by a serous exudate and by a growth of new tissue. Streptococci are present in the fibrin and in the pleural lymph spaces. Extending from the pleura the interlobular septa and the adjacent alveolar walls are similarly thickened, and the alveoli which have become squeezed and distorted in the areas of interstitial pneumonia thus formed show reversion of the epithelial cells to the embryonal type. The alveoli beneath the pleura are emphysematous, and their epithelium cuboidal.

There is a moderate emphysema throughout the non-consolidated areas of the lungs, the alveoli being filled with swollen and fatty epithelial cells, with but few leukocytes, or the alveoli contain a granular material resembling the alcohol precipitate of albuminous fluid. In places the material derived from the broken-down epithelium stains deeply with eosin. The emphysema is considered to be secondary to the obliteration of the bronchi by the bacterial colonies and exudate.

A few small areas composed of cells which have coalesced or become ill-defined, with small round or fragmented, elongated nuclei, are seen in the sections. Such foci resemble the areas of focal necrosis seen in infectious diseases.

Bronchial lymph nodes.—There is a moderate degree of anthracosis. The congestion of the blood-vessels and the capillaries is intense, and there is considerable exudation into the trabeculæ and capsules of the nodes. The centres of reproduction are enlarged, and the proliferation, especially of the small, round, lymphoid cells, is marked. The lymph sinuses are compressed and show but little desquamation of their lining endothelium. No streptococci or other bacteria are demonstrable in the lymph nodes.

In general the lesions consist of an acute exudative and necrotic inflammation of the bronchi and surrounding alveoli; a broncho-pneumonia, associated with a productive inflammation of the framework of the lungs and organization of the alveolar exudate—an interalveolar and intraalveolar pneumonia. Apart from these lesions, there is an interstitial inflammation and thickening of the pleura and interlobular septa, which perhaps is more chronic in character.

Case II. *Clinical History.*—F. F., aged 23, a laborer, entered Roosevelt Hospital, in the service of Dr. Delafield, January 13, 1899. His mother died of pneumonia. He was a moderate drinker. No syphilis or rheumatism. He has had measles, scarlet fever, diphtheria, and

Pott's disease of spine (?). He has had a chronic cough, since his tenth year, with muco-purulent expectoration and night sweats for several years (?). His present illness began about Thanksgiving day, 1898 (?), when he began to lose flesh and strength, and the pulmonary symptoms became more aggravated and were accompanied by dyspnœa. After Christmas, 1898, he became worse, complaining of headache, anorexia and general malaise followed by chills and a febrile movement, profuse sweating, increase of cough and expectoration, and pain in the chest.

On admission: Moderate emaciation and prostration. There was scoliosis of the dorsal vertebræ. Temp. 101° F.; Pulse, 112; Resp. 28. Examination of urine was negative.

Physical examination: Coarse breathing over left lung in front and behind, with mucous râles. Over lower part of right lung, behind, flatness, cavernous voice and breathing, gurgling and mucous râles. In front, breathing is coarse and high pitched, and there are many coarse râles.

In the hospital he became rapidly worse, with frequent cough and abundant expectoration, his breathing rapid and labored, and death followed pulmonary œdema on January 17, 1899.

Autopsy (Dr. E. Hodenpyl), 24 hours after death: The pleuræ of both lungs are covered in places with a fresh layer of fibrin. Both lungs are largely consolidated, the right lower and the left upper lobes being entirely consolidated. The left lower and the right upper lobes present the lesions of a broncho-pneumonia. The bronchi of both lungs show bronchiectatic dilatations, and numerous grayish actinomyces granules adhere loosely to the congested mucous membrane. On section the surface of the consolidated areas appears smooth and is studded throughout with grayish spots, which correspond to the cut sections of the bronchioles or smallest bronchi.

The bronchial lymph nodes are much enlarged and congested. On section, the nodes appear intensely hyperæmic and studded with small grayish foci.

No noteworthy lesions were observed outside of the respiratory organs.

Histological Examination.—This, as in Case I, was limited to the lungs, which were preserved in alcohol. The lesions in the lungs are strikingly similar to those of Case I.

Bronchi.—Many of the smaller and larger bronchi are the seat of a catarrhal inflammation. The bronchial walls are congested and infiltrated by a serous exudate, with polymorphonuclear leukocytes, and the lumina contain leukocytes, fibrin and granular detritus. The adjacent

peribronchial alveoli are filled with leukocytes and desquamated epithelial cells.

The bronchial "granules" or colonies are composed of a felted network of filaments and rods, identical in appearance with those in Case I.

The bronchi are invariably necrotic where the granules are attached, or the necrosis implicates the whole of the bronchus and its adjacent alveoli. The necrotic inflammation is most marked in the walls of the dilatations, where all traces of the normal bronchial structure have disappeared. The cellular exudate in the bronchi adjacent to these colonies is necrotic, the leukocytes being transformed into a granular mass staining faintly with eosin. The largest bronchiectases are about a third of an inch in diameter, fusiform or sacculated, or irregular in outline. The alveoli around the dilatations are compressed and filled with fibrin almost to the exclusion of leukocytes. The fibrin is less compact and dense than in Case I.

Pulmonary parenchyma—The peribronchitic zones of pneumonia present a variety of appearances and consist of alveoli filled with leukocytes and a little fibrin. The alveolar walls are somewhat thickened by a cellular exudate, composed of cells of the epithelioid type, and the network of fibrin encloses a few epithelioid and polyhedral cells. The organization of the fibrin is in places well advanced.

As in Case I there are extensive areas of interalveolar or interstitial pneumonia. A few giant cells apparently formed by coalescence of the alveolar epithelial cells are found in the distorted alveoli enclosed by the new tissue.

The pleura is covered in places with a layer of fibrin and leukocytes, and is considerably thickened by a new growth of œdematous tissue. The blood-vessels are much congested. The exudate on the surface is more or less organized. Throughout the lymph spaces of the pleura and in the fibrin streptococci are found.

The interlobular septa and the alveolar walls adjacent to the pleura and septa are considerably thickened by a new growth of tissue composed of epithelioid and polyhedral cells and a homogeneous or slightly fibrillated basement substance, and the lining epithelium of the compressed alveoli has undergone reversion to the embryonal type.

The alveoli outside of the areas of broncho-pneumonia are somewhat emphysematous and contain large desquamated epithelial cells, which are markedly fatty or vacuolated.

From an examination of a large number of sections, the impression is gained that with the presence of the streptothrix colonies in the bronchi, the catarrhal inflammation is followed by a necrotic one.

No intra-alveolar hæmorrhage has occurred. The blood-vessels throughout the lungs are greatly congested, and the adventitial coats of the large vessels are markedly thickened and œdematous. No thrombosis was noticed, and the intravascular fibrin formation is moderate.

The *bronchial lymph nodes* show the same condition of acute inflammation as in Case I. Scarcely any pigment and no bacteria are found in the sections.

The lesions here, as in Case I, consist of an acute exudative and necrotic bronchitis, with bronchiectasis, a broncho-pneumonia, composed of alveoli filled with a cellular or fibrinous exudate, which is more or less organized, and a productive inflammation and thickening of the framework of the lungs—alveolar walls, interlobular septa and pleura.

The sections of the lungs of both cases are so similar that the cases can be distinguished from one another only by a comparison of several sections. The exudative phases of the inflammation in the bronchi and broncho-pneumonic areas and the proliferation of the alveolar epithelium in the non-consolidated areas are more marked in Case II than in Case I. The interalveolar and intra-alveolar pneumonia and the changes in the pleura and septa are more advanced in Case I. Case II, therefore, represents an earlier and less advanced stage of a lesion identical in its histological characters with that of Case I.

DESCRIPTION OF THE FILAMENTOUS STREPTOTHRIX COLONIES OR GRANULES AND OF THE BACTERIAL MORPHOLOGY OF THE LUNGS.

The gross appearance of the colonies in the bronchi as seen at the autopsies, the bacteriological examination of the coverslips and of the sections, and the cultural findings of the lungs of both cases may for convenience be considered together, as they were similar.

In the larger bronchi of both cases, either free or lightly attached to the congested mucous membrane, were found numerous opaque whitish and soft masses varying in size from 3 to 5 mm. In the bronchi of smaller caliber and throughout the necrotic and consolidated portions of the lungs smaller yellowish-white particles were scattered.

The colonies consist of a network of filaments, whose terminal portions are often bulbous. No distinct bulbs or end capsules were demonstrable in the smears or sections.

Examination of coverslips made from the colonies, stained by aqueous solution of fuchsin and by Gram, reveals an immense number of rods and filaments, with many cocci.

The cocci, nearly uniform in size, are found single, in pairs, or in short chains. They stain by Gram's method even after long decolorization in alcohol. Capsules could not be demonstrated by Welch's Friedländer's or Gram's methods, nor by hot carbolic fuchsin.

The rods and filaments.—The various forms reveal a general similarity in morphology, but vary considerably in length, the shorter forms or rods being 6-10 μ in length, and the longer forms or filaments, two or three times as long as the rods. Many of the rods and filaments are more or less curved, have rough or irregular edges and are pointed or bevelled at one or both ends. Very slender and long filaments staining poorly were found, especially in Case I. The forms with rough edges frequently refuse to stain. The rods and filaments stain by Gram's method, but those which show irregularity in contour often decolorize. All the forms are broader than the tubercle bacillus.

The filaments stain faintly but fairly uniformly with aqueous solutions of methylene blue and of fuchsin. The rods stain irregularly and resemble closely those which were finally cultivated. No branching forms were positively recognized. In the coverslips made from other portions of the lungs the rods are much less numerous and do not predominate over the cocci. To contrast the bacterial morphology of the cases, the filaments on the coverslips of Case II stain more irregularly and are more beaded.

The impression gained after an examination of the coverslips of both cases, is that the rod and filamentous forms are merely variations of one microorganism. No tubercle bacilli were found.

THE BACTERIOLOGICAL EXAMINATION OF THE LUNGS OF BOTH CASES.

Sections.—The streptothrix colonies and cocci are best studied in the sections stained by Weigert's method of staining bacteria.

The filaments have no definite arrangement in the colonies. The streptococci are found in groups or short chains intimately mixed with the filaments. The necrotic cellular exudate in the bronchi contains numerous filaments, many of which are faintly stained and easily overlooked in contrast to the well stained.

A few streptococci and isolated filaments are found in the exudate of the catarrhal bronchitis and in the alveoli filled with fragmented leukocytes and organizing exudate. Where the exudate is mainly fibrinous

or composed of proliferated alveolar epithelium and granular material, no microorganisms are found.

The streptothrix filaments with a few streptococci are present in the necrotic bronchial walls and in the adjacent areas of new tissue, which shows nuclear fragmentation.

Streptococci alone are found in the lymph spaces of the bronchi, interlobular septa, and pleura.

With Sterling's gentian violet the filaments stain intensely and are beaded. In sections stained by this method and by carbolic fuchsin, a similar distribution of the streptothrix filaments is observed.[2]

In the sections of the lungs of both cases stained by Gram's or Weigert's method some of the bronchial colonies, or portions of these, were stained a mahogany or reddish-brown color (Plate XIII, A). The peculiar color reaction is confined to the filaments, the cocci invariably staining blue. The coverslips had been preserved for several weeks before the reaction was observed in the sections and the reddish-brown color fades from the sections in several weeks. The reaction is due to the iodine, as it is found in the coverslips and sections stained and examined in Lugol's solution. Although many of the rods on the coverslips did not give the iodine reaction, it was not confined to any one form of rods. The long, thin, and the irregularly edged filaments stain reddish-brown with noticeable frequency.

The substances known to take this color by iodine solutions are glycogen and the erythrodextrines. Some of the yeast fungi found in healthy stools are also said to stain red. A similar reaction has been noted in certain spore-forming butyric-acid bacilli.

Cultures from the lungs.—In both cases cultures were made at once from the colonies in the bronchi and from various portions of the lungs. Numerous pour- and streak-plates of glycerin and ascites-serum agar were made and grown under aërobic and anaërobic conditions. The ascites serum was slightly alkaline to test paper, the reaction of the glycerin agar was 1.5% acid to phenolphthalein. The original and the dilution plates, none of which were overcrowded, developed colonies which on inspection with a low power appeared identical, and coverslips revealed diplococci and streptococci staining by Gram. The rods were seen only when the solid particles carried over onto the original plates were employed as material for the coverslips. Numerous transplantations

[2] In sections stained by Sterling's gentian violet, the areas of interalveolar pneumonia contain ovoid cells resembling mast-cells and fibroblasts with brilliantly red protoplasmic granules smaller than the eosinophilic granulations of leukocytes.

from the particles (composed mainly of rods), on the original pour-plates, upon fresh media developed streptococcus colonies.

Thus on the ordinary culture media, glycerin and ascites-serum agar and also on Loeffler's blood serum, the streptothrix filaments failed to grow, while from both cases a similar streptococcus with the following cultural characteristics was alone isolated: Broth becomes turbid with a moderate whitish deposit resembling a pneumococcus broth culture more closely than a short-chained streptococcus. On potato no growth at 37° C. In gelatin stabs isolated colonies develop slowly along the puncture. Smears made from the broth culture reveal short-chained streptococci and diplococci. Subcutaneous inoculation of the broth streptococcus cultures and of the pus and colonies of the lungs of both cases in mice were negative.

INOCULATION OF ANIMALS WITH THE STREPTOTHRIX COLONIES AND PUS FROM CASES I AND II.

CASE I.—Three rabbits were injected intratracheally with a broth suspension of the colonies and pus. Two of the rabbits were killed two weeks later, with negative post-mortem results.

Rabbit, No. 3805.—This, the third rabbit, was injected with a small syringeful of the suspension on November 11, 1898, and died February 25, 1899, 63 days after inoculation. The animal is moderately emaciated. Autopsy reveals an extensive empyema of the left pleural cavity, the lung being compressed against the spinal column. The pus has a strong odor, and contains small whitish granules. Coverslips reveal cocci and numerous shorter and longer filaments, similar in morphology to those injected. Glycerin-agar pour-plates planted with the pus of the empyema, yield pure cultures of streptococcus. The mediastinal lymph nodes are enlarged, whitish and cheesy in appearance. Throughout the right lung, numerous whitish foci are scattered; the pleura appears normal. The other viscera are congested. The lungs were distended and preserved in alcohol.

Sections of the *right lung* show a general catarrhal and suppurative bronchitis and large peribronchitic zones of pneumonia. The exudate in the bronchi and alveoli is composed almost exclusively of leukocytes, and contains numerous streptothrix clumps. In the hæmatoxylin and eosin sections the clumps stain deeply with eosin, a few threads or filaments towards the centre being stained by hæmatoxylin. Stained by Weigert's or Gram's method, and by carbol fuchsin (Weigert), the peripheral filaments are seen to possess terminal swellings, and are radially

arranged, converging towards the centre of the clump. The rather dense zone of leukocytes attracted by chemotaxis around the clumps shows marked nuclear fragmentation. No streptococci or clusters of well-defined filaments are present in the lung. The clumps are smaller and do not resemble the streptothrix colonies seen in the bronchi of the human lungs, nor were such clumps seen in these lungs.

The pleura of the *left lung* is covered with a thick layer of necrotic exudate, containing small clusters, streptothrix filaments and streptococci. The pleura is much thickened by new tissue which extends into the adjacent and compressed lung tissue. A large cavity in the upper lobe is filled with a necrotic exudate. The wall of the cavity is composed of rapidly proliferating granulation tissue and of a fibrous zone of well-formed but œdematous connective tissue. The lung tissue around the cavity is compressed, and the alveolar epithelium shows marked reversion to the embryonal type.

There is a diffuse broncho-pneumonic consolidation, mainly of leukocytes, which exhibit marked nuclear fragmentation. There is a general suppurative bronchitis. Numerous streptothrix clumps are present in the exudate. Streptococci and groups of well-stained filaments are less frequently seen. The alveolar walls outside of the areas of consolidation are thickened by a cellular exudate, composed of polymorphonuclear leukocytes and many polyhedral cells or fibroblasts.

The intima of the larger and smaller blood-vessels is swollen, and there is considerable proliferation of its cells. The endothelium is well preserved. The muscular coats of the arteries have undergone hypertrophy, and the adventitia is thickened and œdematous.

The liver sections show a marked round-cell infiltration in Glisson's capsule. There is parenchymatous degeneration of the kidney and congestion of the spleen.

With the empyemal pus a second rabbit was injected intraperitoneally, February 25, 1899. Two weeks later it seemed ill and was emaciated; as death did not intervene, and the animal was recovering, it was killed April 6, forty days after inoculation. The autopsy showed a few whitish recent cicatrices on the peritoneum. With this rabbit, our last chance of isolating the streptothrix of the first case was lost, since as above stated, we had failed to secure a growth on artificial media.

Case II.—Two guinea-pigs were injected in the peritoneum and two rabbits, one intravenously, the other through the trachea, on January 17. The bronchial material for all the injections was suspended in 1 cc. of broth.

Guinea-pig No. 3591 died January 24 with a general suppurative peritonitis. Many adhesions between the coils of intestine and retraction of the omentum are present. The mesenteric lymph nodes are enlarged, yellowish in color, the cross-section of the nodes resembling that of cheesy tuberculous nodes. Numerous small streptothrix granules are found free in the exudate or adhering to the serosa, and the examination of coverslips made from the pus reveals numerous cocci, and longer and shorter filaments and thin rods. Glycerin-agar pour-plates yield streptococci alone. The lymph nodes contain streptococci and filaments.

The *second guinea-pig* died January 24. In addition to a suppurative peritonitis, a serofibrinous pericarditis is found. The lungs and pleura appear normal. The viscera show intense congestion.

A rabbit injected intraperitoneally on January 24 with the pus of the first guinea-pig, was killed April 6. The autopsy was negative.

Rabbits.—A large rabbit, as above noted, was injected through the trachea on January 17, 1899, and killed January 30. Autopsy reveals an empyema of the right pleural cavity. The right lung is compressed and whitish in color and covered by fibrin. Examination of coverslips made from the pus show cocci, and filaments resembling in morphology those injected. The lungs are distended and hardened in alcohol. The pericardial layers are adherent. There is marked œdema of the mediastinal tissues, and the lymph nodes are enlarged, whitish and soft.

The left lung is studded with whitish foci. The other viscera are normal, except for coccideal areas in the liver. Sections of the left lung present a general catarrhal bronchitis, with small round-celled peribronchitic infiltration, mostly localized in areas resembling Arnold's lymph nodules. The whitish foci correspond to areas of broncho-pneumonia.

In the atelectatic or compressed portions of the *right lung*, the alveolar walls are thickened by a cellular exudate composed mainly of leukocytes, and by capillary congestion. There are extensive zones of peribronchitic consolidation, the alveoli and bronchi being filled with leukocytes. The leukocytes of the alveolar exudate have undergone nuclear fragmentation and the alveolar walls are in places infiltrated with leukocytes. Streptothrix clumps and a few isolated filaments with numerous streptococci are present in the exudate. The pleura is covered by a necrotic layer of fibrin and leukocytes, containing clusters of streptothrix and numerous streptococci. Beneath the thickened and necrotic pleura the lung tissue is compressed, and deeper a well-marked zone of new tissue has formed.

Sections of the heart and pericardium show a fibrinous and adhesive pericarditis; the fibrin has undergone hyaline metamorphosis and the pericardial layers and adjacent muscular fibres are necrotic, and infiltrated with leukocytes. Here and there in the myocardium beneath the necrotic layer, a well-marked round-celled infiltration exists. Streptococci are present in the exudate.

The blood-vessels throughout the right lung show more or less marked round-celled infiltration of their adventitial coats. The intima is regularly swollen, and in some arteries a well-marked obliterating endarteritis is made out.

Rabbit No. 3727, the second above mentioned, was injected January 17 in the ear vein with bronchial material of Case II and died February 14. Animal is much emaciated. The left pleural cavity is filled with whitish pus of the consistency of cream. The lung is compressed, the upper lobe being adherent to the ribs. The right pleural cavity is partly filled with a more fluid pus. The pleuræ of both lungs are covered with a layer of fibrin and pus. The other viscera of the rabbit are normal.

Coverslips made from the pus reveal cocci and filaments identical in morphology with those injected. Glycerin- and serum-agar planted with the pus yielded pure cultures of streptococcus. Numerous potatoes planted with the pus remain sterile. The organs of a rabbit were planted with the pus: on the kidneys streptothrix colonies developed, which are described in detail below under methods of isolation.

Histological examination.—The pleura is covered with a layer of necrotic fibrin and leukocytes with numerous streptothrix clusters. The superficial part of the thickened pleura is necrotic and infiltrated with leukocytes. The lung tissue adjacent to the pleura is compressed and infiltrated with small round-celled granulation tissue, extending in the lung to a variable depth from the surface and giving rise to areas of interstitial pneumonia. The alveoli enclosed in these areas assume bizarre shapes, and the alveolar epithelium may become columnar. Most of the sections of both lungs contain several larger and smaller abscesses and as no trace of bronchial structure remains even about the small abscesses, we are inclined to consider them metastatic abscesses.

Streptococci and clumps or clusters of ill-defined filaments are seen in the pleural exudate and in the abscesses where they are much less abundant. The walls of the cavities are formed by a layer of necrotic fibrin. Round-celled granulation tissue extends from this zone directly into the adjacent lung.

There is some dilatation of the bronchi, which in general show only

moderate degrees of catarrhal inflammation. Considerable intra-alveolar hæmorrhage has occurred about the abscesses. In some sections, the pneumonic exudate is extensive, and the infiltration of the alveolar walls has reached a moderate degree. The disintegrated tissue near the necrotic exudate abounds in nuclear fragments, and irregularly staining detritus, which in places have been taken up by large vacuolated or fatty cells.

A few circumscribed nodules composed of epithelioid cells, mostly disintegrated and with fragmented nuclei, occur here and there. Giant cells are not seen.

As regards the bacteriological examination of the sections, one is struck by the scarcity of the streptococci, and although the peculiar homogeneous clumps occur in moderate numbers, clusters of well-defined, or isolated filaments are but rarely seen. The clusters of filaments become transformed into the homogeneous eosinophilic clumps, which stain intensely with Weigert's fibrin method or by Gram. Transitional forms between the clusters of well-defined filaments and the homogeneous clumps occur. The most common one is a granular mass barely staining with hæmatoxylin or with anilin gentian violet, in which, however, a few filaments are definitely recognizable. Such transitional forms were found in all the experimental streptothrix lesions.

Besides the changes of the blood-vessels described in the previous rabbit, there is a general and well-marked hypertrophy of the muscular coats of the arteries throughout the lungs. The liver and kidney show acute congestion and slight parenchymatous degeneration.

The empyemal pus of the previous rabbit (3727) was injected into the ear vein of a rabbit (3752) and into the peritoneum of another rabbit (3806).

Rabbit 3752, intravenous injection of 1 cc. of the pus. Animal died 72 hours after injection.

Autopsy.—The peritoneum is intensely congested, and contains straw-colored fluid. The omentum is œdematous. The liver is congested and mottled. Kidney and spleen are congested. Through the pleura numerous small foci are seen. No cultures were made, as the autopsy was held 24 hours after death. The cause of death is considered to have been an infection with the streptococcus, perhaps increased in virulence by passage through the first rabbit.

Histological examination.—The lungs are highly congested. The blood-vessels in places contain the injected material. The emboli are composed of detritus, necrotic fibrin and leukocytes, with streptococci,

and a few homogeneous streptothrix clumps surrounded by a zone of leukocytes. Fibrinous thrombi, in places necrotic and without evidence of organization, occlude the blood-vessels. The coats of the blood-vessels are the seat of a necrotic inflammation extending to the adventitia, which is infiltrated with a serous exudate.

In the neighborhood of the thrombosed blood-vessels the alveoli are filled with fibrin, detritus and leukocytes, forming small areas of lobular pneumonia throughout the lungs. The numerous small white foci found at the autopsy, correspond to groups of several alveoli filled with leukocytes. In the larger areas the disintegration and infiltration with leukocytes of the alveolar walls are more advanced, the consolidation being composed of a mass of leukocytes, shrunken round cells and nuclear fragments, with necrotic fibrin. The liver contains numerous large areas of focal necrosis. The liver and kidney show parenchymatous degeneration, congestion and bacterial capillary emboli. There is an acute hyperplastic splenitis.

Rabbit 3806 was injected into the peritoneum with 1 cc. of the empyemal pus of rabbit 3727, on February 14 and died eleven days later. There is a general suppurative peritonitis, the coils of the intestines being matted together with fibrinous adhesions. The pus contains numerous cocci and filaments. The serosa of the liver, spleen and diaphragm is covered with a thick layer of fibrin. The mesenteric lymph nodes are enlarged, whitish and cheesy on cross section. The spleen is studded with small whitish foci.

Beneath the pleura of the lungs, numerous smaller and larger whitish areas are seen, the pleura itself appearing normal. The upper lobes of both lungs are completely, the lower lobes irregularly, consolidated. The upper left lobe contains a large cavity filled with whitish material and blood (Plate XII, Fig. 6). The bronchi of the right upper lobe are greatly dilated and contain pus, the consolidation of the lobe being whitish in color.

Histological examination.—The pathological process in the lungs presents the same phases of inflammation as in the lungs of the rabbits injected through the trachea, but the topography of the lesions is different, corresponding to their hæmatogenous origin.

The consolidated lobes contain wedge-shaped abscesses and areas of infarction, composed of alveoli more or less filled with leukocytes, fibrin, and a necrotic material, surrounded by a dense zone of leukocytes (Plate XII, Fig. 7). At the apex of the wedges the central blood-vessel is obliterated by a fibrinous thrombus which is necrotic and infil-

trated with leukocytes. The necrotic inflammation extends through the coats of vessels into the surrounding tissues, which are disintegrated and filled with nuclear fragments. A layer of necrotic fibrin surrounds the zone of pus cells, and the adjacent lung tissue is infiltrated with small round cells, an early stage of limitation or demarcation by fibrous tissue.

Numerous smaller abscesses without apparent connection with the blood-vessels are scattered throughout the lung. The upper lobes, are diffusely consolidated, the bronchi are markedly dilated, and the seat of a mild catarrhal bronchitis, with desquamation of the epithelium. In the lower lobes, there is broncho-pneumonia with suppurative bronchitis. The tissue surrounding the largest bronchiectases, is extensively infiltrated with blood.

The coats of the blood-vessels are the seat of an exudative inflammation most marked in the adventitia, and fibrinous thrombi obliterate some of the vessels. There is a general round-celled periarteritis of the smaller vessels. New tissue is found around the bronchial dilatations in the form of areas of interstitial pneumonia. The lungs in other situations are atelectatic and the alveoli contain desquamated cells and granular material.

The serosæ of the liver and spleen are thickened and covered with a layer of fibrin and pus cells. Numerous streptococci, a few clumps and clusters of streptothrix, and isolated filaments which give the reddish iodine reaction are found in the layer of exudate; the liver and kidneys show acute congestion and a moderate degree of parenchymatous degeneration.

The Malpighian bodies of the spleen are enlarged, the blood-vessels are congested, and contain many leukocytes. The endothelial cells of the capillaries show proliferation and degeneration, and a fibrinous exudate is found beneath the capsule.

To summarize briefly, the introduction into the lungs of rabbits through the trachea of the bronchial material of Case I and Case II, consisting of streptococci, streptothrix rods, pus cells, etc., was followed by suppurative and necrotic broncho-pneumonia with bronchiectases, pulmonary abscesses, and extension of the inflammation to the pleura and pericardium with empyema.

Inoculation of the infectious bronchial material of Case II into the ear vein of a rabbit gave rise to pulmonary abscesses, diffuse pneumonic consolidation of the lungs and empyema. The empyemal pus

of this rabbit injected into the peritoneum of another rabbit produced a suppurative peritonitis with metastatic pulmonary abscesses.

In guinea-pigs, inoculation of the bronchial material of Case II into the peritoneum produced suppurative peritonitis.

In the rabbit the pathological process was acute, the leukocytic infiltration and disintegration of tissue led to the formation of abscesses with well-marked limitation of the process by a zone of demarcating connective tissue.

THE METHODS OF ISOLATION, AND THE MORPHOLOGICAL AND BIOLOGICAL CHARACTERS OF THE STREPTOTHRIX OF CASE II.

Methods of isolation.—The usual laboratory media having been found unsuitable for the cultivation of the streptothrix, the method of streaking the fresh organs of rabbits was resorted to. The organs of normal rabbits, immediately after killing, were transferred to Petri dishes, precautions being taken to avoid contamination during removal. The kidneys were halved. The various organs were planted with the empyemal pus of rabbit 3727 and the Petri dishes placed in covered chambers containing sterilized water to prevent drying of the organs. After two days at 37° C., six elevated whitish colonies, the largest 2-3 mm., developed on the kidneys. On the other organs—liver, spleen, heart and muscle—no growth was visible, but streptococci were found on the coverslips, and recovered in pure culture from these organs. Coverslips prepared from the colonies on the kidney showed rods resembling in morphology the bacillus of diphtheria, mixed with moderate numbers of cocci. The average length of the rods approximates that of the tubercle bacillus; they are somewhat broader than this bacillus. The ends are frequently bulbous, less often distinctly wedge-shaped. Some of the rods are frayed out at the end, an appearance suggestive of fragmentation and not common to bacilli. No indication of branching is found, although it was searched for. Stained with methylene blue, the rods appear beaded or striated. The staining reactions and morphology of the growth on kidneys will be more fully described below.

The kidney growth was planted in broth and on the various organs

of a fresh rabbit. The broth tubes yielded pure cultures of the streptococcus. Again on the planted halves of the kidneys, but on none of the other organs, a growth of rods was obtained. Successive transplantations upon fresh kidneys, and also on the other organs, of rabbits were then made at intervals of three days. In no case did the rods grow, as determined by smears, on any organ but the kidney. A photograph (Plate XI, Fig. 5) shows colonies of the streptothrix growing on the rabbit's kidney.

The second generation on kidney was an abundant one, numerous colonies developing along the course of the streaks. Coverslips of the growth revealed the rods, morphologically speaking, in pure culture. Broth, however, planted with the growth again yielded a pure streptococcus culture. The third generation on kidney was still more luxuriant, the growth forming confluent and raised masses, glistening slightly, and resembling in texture the substance of yeast cakes. The later generations on kidneys were moister and diffusely scattered over the surface. The morphology of the third and later generations differed somewhat from that of the first growth, in that the rods were longer and more frayed out at the ends. The wedge-shaped rods were observed only in the early kidney growths.

The striking predilection of the rods for the kidney as a medium of cultivation, and the complete absence of growth on the other organs of the rabbit, is a fact of singular importance. Many observers have recorded observations on the special suitability of various organs for the growth of a variety of microorganisms, but so far as our knowledge extends, no such striking predilection for a particular organ has been noted.

After several generations on kidney had developed we were somewhat perplexed how to proceed further in our study of the rods which were found later to be a species of streptothrix. The streptococcus was still present as revealed by broth cultures in the third successive kidney growth, and the rods still refused to grow in broth, at least in symbiosis with the streptococcus. The growth of the streptothrix on kidney being assured, methods for separating the two microorganisms engaged our attention. Three methods were tried: First, by taking advantage of a

possible difference in the thermal death point of the two microorganisms. The growth on the kidney was suspended in a number of broth tubes which were subjected, some to a temperature of 50° C., others to 56° C. for ten minutes. A number of trials at both temperatures were made. Both microorganisms failed to survive the exposure, transplants failing to produce a growth on broth and on kidney. The second method, prolonged cultivation from kidney to kidney, the transplants being made after three days' growth at 37° C., we were led to believe would succeed, from a comparison of the coverslips taken from the early generations on kidney after several days' growth at 37° C. and later when the kidney growth was kept at room temperature. It was observed that the rods overgrew the streptococcus at incubator temperature, the coccus first becoming visible on the smears when the cultures were kept at room temperature, when the rods, as was determined later, cease to grow below 28-30° C. approximately. The sixth generation on kidney controlled by broth cultures was a pure streptothrix culture. The continuous kidney cultivation was forced upon us, as at first the rods failed to grow in our broth, a fact which did not encourage us to try the next and third method of separation by washing the mixed kidney colonies, which, however, led to success. A well isolated colony was looped, and shaken in successive broth tubes. Five broth tubes and kidneys were finally planted with the washed bits of growth. The kidneys and one of the broth tubes yielded pure cultures of the streptothrix; one tube gave a mixed culture; the others, a pure streptococcus culture. The broth used for all came from the same stock of beef peptone broth, 1.5% acid to phenolphthalein.

The first broth growth was meagre, and with it glycerin and serum agar plates and broth tubes were planted. Again the broth alone showed a growth. Anaërobic stabs in four raw eggs were tried. In one egg several masses composed of a felted clump of mycelium developed but the cultivation in eggs was considered uncertain and was abandoned.

The persistent refusal to grow on any organ but kidney, or on solid media, had suggested the advisability of trying broth and agar in which kidney infusion replaced the usual meat infusion. Broth and 1.5% agar were made both with human and lamb kidneys, with the usual addition of peptone and salt. The reaction was found neutral to test paper or about 0.4 acid to phenolphthalein. In plants of the media with the mixed growth on kidney the streptococcus alone developed.

It was thought possible that, as in the case of the gonococcus, uncoagulated proteids were essential for growth. Chopped rabbits' kidneys were

added to fluid agar at 43° C. planted and poured. The streptothrix failed to grow, however. The following media were also planted, all with negative results: Agar and broth plus sterilized urine; ascitic serum agar, slightly alkaline, with and without glycerin; Loeffler's and coagulated calf's blood serum; prune juice media with peptone and salt of different reactions, alkaline, neutral, and slightly acid; semi-solid mixtures of gelatin and agar, and Hiss's typhoid plating medium.[3]

Broth cultivation of the streptothrix was pursued for two months, transplants being made at intervals of a week, the growth becoming more vigorous and abundant in each successive culture.

Plating media.—From the eighth generation on broth a growth was finally obtained on solid media—the usual peptone agar with addition of 5% glycerin, made to react neutral or 0.5% acid to phenolphthalein.

Separate portions of the agar media with and without addition of 2% glucose were made to react 0.5 alkaline, neutral, and 0.5 to 1% acid to phenolphthalein. As the acidity is decreased, the plating media become softer. To insure accuracy, each set of media was tested after the final sterilization, and two original and one streak plates were planted with large loopfuls of the eighth generation on broth.

In the first and second series of plates those of 0.5 acid reaction furnished the most abundant growth, the other plates containing only several colonies. In a third series the most abundant growth was obtained on the plates of neutral reaction; several colonies now grew on the media of 1.5% reaction.

At the maximum only 50 to 60 colonies developed on the original plates, in marked contrast to the overcrowded pour-plates planted with bacterial cultures.[4]

Description of the streptothrix colonies on neutral or 0.5 acid glycerin agar.—The colonies are visible at the end of 48 hours at 37° C., and attain their maximum development in the course of a few days. The largest are 3 to 4 mm. in diameter. The growth resembles more nearly that of bacteria than the dry growth described as characteristic for many

[3] Hiss, *Journal of Experimental Medicine*, 1897, ii, p. 677.

[4] The following explanation of the scanty growth on solid media is suggested: Though spores were never observed, the sporulation of the streptothrix in the broth culture may be reduced to a minimum, and thus have escaped observation. The colonies may arise only from the germination of the few spores and the subsequent rapid increase in length of their mycelia; whereas the non-spore-bearing streptothrix rods, which are in the majority after the fragmentation caused by the transfer from broth to solid media, stop growing, or grow so slowly that no colonies visible to the naked eye or under a low power develop. However this may be, solid media, barring the kidney, are not suitable or favorable soil for this streptothrix.

of the Streptothrices and allied microorganisms, the tubercle bacillus, etc.

The deep colonies are pin-head or smaller in size, whitish in color and opaque. Spreading colonies are formed out of these deep colonies by extension of the growth into the medium, or on the bottom of the plate. The borders of these so-called disseminated colonies are made up of smaller colonies or groups of rods which lend them a characteristic appearance. The central opaque portions of the colony resemble the non-spreading deep colonies.

Stab cultures on agar (1.5% acid reaction) planted from the colonies now yielded a fairly abundant growth of discoid and isolated colonies along the puncture, the largest ones being 2 to 3 mm. in diameter, whitish in color and sharply defined.

A scanty, whitish growth was now obtained on coagulated ascitic serum containing broth and sugar, and on Loeffler's blood serum. The colonies adhere firmly to these media in contrast to the loose growth on the kidney and on moist glycerin agar. Firm adherence to the surface of media is characteristic for many species of streptothrix, being due to the penetration of the growth into the depth of the media.

The growth in *broth* resembles the granular variety of a streptococcus culture, the small discoid granules adhering firmly to the sides of the tubes, leaving the broth clear. The differences observed between the early and the later broth cultures are due to the more abundant and rapid growth of the streptothrix as cultivation proceeded. In the later cultures a sticky sediment was constantly present after a few days of growth, the granules becoming larger and fluffy with the increase in size and then sinking to the bottom after slight handling of the tubes.

Two varieties of broth culture occur, the one described above with small, hard and adherent discoid granules, or larger fluffy particles; the other the large clumpy variety of broth culture. The clumpy variety is characterized by the formation of a few large clumps at the bottom of the tube which slowly increase in size; the clumps are dull gray in color, firm and gritty. It was observed in a few of the early broth cultures and later in some broth tubes, which were planted with the cheesy material of the lymph nodes, etc., of rabbits that had been injected subcutaneously with pure cultures of the streptothrix. The clumps are seen to form directly from the bits of cheesy material, which slowly increase in size after 8 to 12 days in the incubator at 37° C.

Fermentation tubes.—In litmus 2% grape-sugar broth, no gas forms. The growth develops equally well in both arms of the tube. The color

begins to change 24 to 48 hours after the growth first becomes visible, the production of acid being slow. Discoloration soon occurs and is most marked in the closed arm. Grape sugar does not increase the rapidity of growth in broth. Other sugars were not tried.

Milk cultures.—In litmus milk after 48 hours, the color changes, soon becoming markedly red. Clotting usually occurs on the fourth day or may be delayed to the fifth or seventh day. The clot is soft, finely divided and occupies the whole of the fluid. The purity of the milk cultures was tested by transplants into broth.

In order to determine the cause of the clotting broth cultures were filtered through sterilized filters and added to sterilized milk. No coagulation of the milk occurred after addition of a few drops up to several cc. of the filtered broth culture, either at once or after several days at 37° C. The coagulum is apparently caused by acid production, no soluble enzymes being demonstrable.

Several determinations of the acidity of broth and milk cultures, after 7 to 10 days' growth, were made. Broth was found to have increased in acidity from 1.5% acid to 4.5% and 6% acid to phenolphthalein.

In Dunham's peptone solution of various degrees of reaction no growth occurs, and on plain or glycerinated potatoes, tested and found to be only a few tenths of one per cent acid, no growth occurs, as confirmed by smears.

The absence of growth on potato is not in consonance with the well-recognized suitability of the potato for the cultivation of many Streptothrices. Potato culture is said to favor the formation of the aërial and spore-bearing hyphæ of a number of species of this genus (Streptothrix Eppingeri), and a characteristic musty or mouldy odor accompanies the sporulating stage of growth. Streptothrix Israeli (?) and a few other species fail likewise to grow on potato.

Vitality.—The streptothrix is short lived. The longest period it was found capable of retransplantation from broth to broth was three weeks. Grown on kidney at 37° C. for four days, and then kept for two weeks in a moist chamber at room temperature, when further growth ceases, successful transplantation on fresh kidney becomes uncertain.

Optimum temperature.—It grows best at 37° C. In the summer months when the temperature ranged at times from 24° C. to 29° C., a few tubes of broth and gelatin showed a slight growth on the fifth or sixth day.

In broth, the streptothrix grows equally well in the absence or presence of oxygen, and no change in morphology was observed in the anaërobic growth. The growth on all media is odorless.

Morphology of the streptothrix.—The morphology of the streptothrix is well shown in the photomicrographs (Plate XI). The streptothrix on the coverslips made from the bronchial colonies and pus, and from the lesions and pus of rabbits and guinea-pigs induced by these, presents rod-like or filamentous forms; no branching forms or bulbs, after appropriate staining or in the fresh condition, were identified.

In the growths on the rabbit's kidney, it still retains its rod-like form, but is somewhat shorter, and closely resembles bacilli belonging to the pseudo-diphtheria group. The club-shaped or wedged ends and bulbous central portions of the rods stain intensely with the dyes, and with aqueous solution of methylene-blue marked metachromatism is present. The grouping in closely approximated and parallel lines is frequent. In the later generations on kidney the ends of the rods become poorly defined or taper off gradually, and stain faintly, the first indications of fragmentation (Plate XI, Fig. 1).

The irregularity in staining with methylene blue and aqueous fuchsin solutions gives rise to a beaded appearance. When dilute solutions of dyes slowly penetrate under the cover glass, the bulbous ends and the round refractive swellings stain more intensely than the rest of the protoplasm; in some places the rods remain unstained, or only a portion of the refractive swellings may stain. The bulbous ends stain deeply with Delafield's hæmatoxylin, whereas the rest of the protoplasma is scarcely tinged.

With P. Ernst's stain (methylene blue and Lugol's solution), every rod possesses one or several intensely black, round spots; these are usually single, situated at one extremity or at both ends, or irregularly distributed in the protoplasm, which stains a faint yellow (Plate XI, Fig. 4). These bodies are often smaller in diameter than the breadth of the rod.

No branching forms were discovered in the growth on kidney, either in the coverslip preparations or in the sections of the growth on kidney. Branching was first positively recognized in the early generations in broth (Plate XI, Fig. 4), the fragmentation of the filaments and the branching increasing with the further cultivation in broth.

As shown in the photographs there are striking differences in morphology obtained after different staining methods. Figures 2 and 3 (Plate XI) are of smears made from the same colony on glycerin agar, stained respectively by methylene blue and by Gram. Figure 2 shows the beading of the filaments and large numbers of small rods or coccus-like forms. In Figure 3, from specimens stained by Gram, these features are not prominent, the staining being diffuse, intense, and only a few

short rods are seen. The contrast in the morphology[5] after the two stains is so striking that we believe many have fallen into error when describing the morphology of various streptothrices and have laid too much stress on such coccus-like forms, which have been often mistaken for spores. By careful focussing the connecting links between the colored portions of the rods (stained by methylene blue) can be recognized. Stress is laid on these observations on account of the frequent neglect of most observers to mention the stains employed.

The viscid deposit in the broth tubes seems to be largely intracellular substance, mixed with rods which stain faintly. Curious forms resembling stalks were observed when this material was examined. They varied greatly in size, and their branches form an intricate network, the meshes of which vary in size from delicate filaments up to very broad stalks.

When stained by methylene blue or by P. Ernst's method, the relative position of the darkly-staining spots in the stalks to each other would seem to indicate that stalk formation occurs by the coalescence of the filaments along their length. Sauvageau and Radais, who suggest this explanation, give an excellent description of similar stalk formations in the two species of streptothrix they described.

Our streptothrix in broth and milk cultures exhibits extreme pleomorphism. There are short rods with bulbous extremities, longer rods with marked beading, or with considerable unstained protoplasm. In milk the short rods, and those with bipolar staining are especially common; and long, thin and curved filaments, with or without irregular beading, resemble the filaments of the bronchial colonies.

The short rods with bipolar staining from the milk cultures closely resemble those recovered from the cheesy lesions of rabbits after subcutaneous injection with pure cultures. The scarcity of such forms in the lesions, and the difficulty of recovering the streptothrix in cultures, unless large loopfuls of the cheesy material are transferred, incline us to regard these as degenerative forms.

Morphology of the streptothrix in the experimental lesions.—In the lesions of the guinea-pigs and rabbits produced by the inoculation of the bronchial material of Cases I and II, the streptothrix was present in large numbers in the pus of the pleura and peritoneum together with numerous streptococci. In morphology the rods and filaments resembled closely those composing the streptothrix colonies of Cases I and II.

[5] Bostroem with the Actinomyces bovis and Aoyama with the species of streptothrix which he has lately isolated, have called attention to this contrast in size presented by streptothrix after various staining methods.

In the pulmonary lesions produced by intratracheal introduction of pure cultures into rabbits the streptothrix is found in clumps, which undoubtedly arise from the discoid granules of the broth cultures, and only a long search reveals a few isolated filaments. In the lesions of the skin or lymph nodes after subcutaneous injections a few clusters of streptothrix filaments or rods with bipolar staining may be found.

The clumps stained by hæmatoxylin and eosin are strongly eosinophilic and homogeneous. The fine radiations which extend from the periphery to the centre of the clump when stained by Mallory's [6] method or by carbolic fuchsin (Weigert) appear as long and variously-shaped bulbs or terminal swellings, which extend into the zone of leukocytes surrounding the clump. In every clump a few distinct bulbs enclosing central well-defined mycelial filaments are found.

In contrast to their faint staining by these methods, the bulbs and terminal swellings stain intensely by Weigert's fibrin-stain (Plate XIV). As a few clumps were found in the bronchi and fibrinous pleural exudates of the rabbits injected with the bronchial material of Cases I and II the streptothrix filaments under certain conditions evidently become transformed or agglutinated into clumps, similar to those derived from the granules of the broth cultures. The few finely granular clusters of more or less disintegrated and faintly-staining rods seem to be transitional forms which may later develop into clumps.

The intense staining of the terminal swellings of the mycelial filaments in the clumps when stained by Weigert is well shown in our drawing (Plate XIV). They closely resemble the bulbous forms of the tubercle bacillus as depicted by Babes and Levaditi in their article on the actinomycotic form of the tubercle bacillus and also by Lubarsch and by Schultz in their articles on the bulbous forms of the tubercle bacillus and other bacteria. For a discussion of the nature of the bulbs, etc., which cannot be entered upon here, we refer to Lubarsch.

The morphology of the streptothrix remains the same when grown in collodium sacs within the peritoneal cavity of the rabbit.

The original smears of the bronchial colonies had been kept for several weeks before being stained by P. Ernst's method and then no chromatic particles were seen. To determine whether this was due to prolonged drying of the smears, even after the usual fixing, smears from broth cultures were kept after fixing by heat for several weeks. They were then stained with hæmatoxylin, by Ernst's method, and by

[6] Mallory and Wright, Pathological Technique, p. 282. Method No. 1. Philadelphia, 1897.

methylene blue. The rods invariably stained faintly, and no granules were found. Prolonged drying changes apparently the staining properties of the plasma. Such observations, however, do not aid in determining whether the granules are nuclear or merely reserve depots of material, but perhaps explain their absence from the original smears.

Curious forms characterized by marked tenacity for retaining the stain after decolorization in alcohol were observed in the later generations on broth. When the smears were stained by hot carbolic fuchsin and decolorized in 97% alcohol, followed by washing in water and drying, the intensely staining forms are bulbous, less often bacillary or coccus-like, or branched (Plate XIII, B). With a methylene-blue afterstain the rods stained blue and these questionable forms retain their brilliant red color. When weak solutions of acids are used, the decolorization is prompt and complete.

The morphology of the streptothrix growing on various solid media, Loeffler's and coagulated blood serum, etc., does not differ from that described above.

Growth in the hanging drop.—The streptothrix is non-motile. The branching of the delicate mycelial threads is seen to best advantage in the hanging drop. The irregular swellings and club-shaped ends of the rods, and the round refractive bodies, some apparently free, are also well observed.

The difficulty of watching the growth was increased, as the streptothrix does not grow at room temperature. In broth, acrogenous spore formation was not observed nor was actual fragmentation seen to occur. We were able to watch the actual increase in length of the small buds or projections from the rods and filaments into branches of considerable length. The observations entitle us to consider the branching a real one, and to separate definitely our streptothrix from the group of so-called pleomorphic bacteria. The trend of recent studies limits the usage of this epithet, to describe not a group, but merely a condition of pleomorphism in microorganisms.

The vast number of branching forms seen in smears made from pure cultures also justify us in considering our rods as a species of the genus Streptothrix.

EXPERIMENTAL LESIONS PRODUCED BY INOCULATIONS OF PURE CULTURES OF THE STREPTOTHRIX.

SERIES I.—The eight rabbits of this series were injected on March 27, 1899, either with the pure growth of streptothrix of the 6th generation

on kidney, suspended in broth, or with broth cultures obtained by washing the 3d generation on kidney, as indicated below.

Intraperitoneal injections: Small rabbit No. 1.—Kidney culture. The animal, moderately emaciated, was killed 42 days after inoculation. Whitish patches of thickening of the parietal peritoneum are found. In places the patches are depressed. Autopsy otherwise negative.

Small rabbit No. 2.—Broth culture. Animal emaciated, was killed 42 days later. Whitish patches somewhat more depressed but otherwise similar to those of rabbit No. 1, are found. Microscopic examination shows the patches to be formed of new fibrous tissue. Pea-sized yellowish nodules are found attached to the intestine and mesentery, and coverslips prepared with the dry material reveal a few rods with a faint bipolar stain, but broth tubes planted with a loopful remain sterile. The cæcum contains coccidial ulcers which extend down to the peritoneal coat.

We believe the lesions described to be late or nearly healed stages of lesions produced by the streptothrix. Both animals received small quantities of culture.

Rabbit No. 3.—To compare the lesions following upon combined injections of the streptothrix and the streptococcus, with those produced by the streptothrix alone, a rabbit was injected in the peritoneum with 1 cc. of a (7th day) broth culture of the streptococcus isolated from Case II, together with the broth streptothrix culture. Animal killed 44 days later; no lesions were found.

Intravenous injections: Rabbit No. 4.—Kidney culture of the streptothrix. Animal killed 43 days after inoculation. Autopsy negative.

Rabbit No. 5.—Broth culture. Killed 43 days after inoculation, autopsy likewise negative.

Intratracheal injections: Rabbit No. 6.—Kidney culture. Killed 44 days after inoculation. Autopsy negative.

Rabbit No. 7.—Kidney culture. Killed 49 days after inoculation. Autopsy negative.

Rabbit No. 8.—Broth culture of streptothrix and streptococcus. The possibility that the streptothrix alone introduced into the lungs through the trachea might produce no lesions, led us to inject 1 cc. of a (15th day) broth culture of the streptococcus isolated from Case II, together with a broth streptothrix culture, into the trachea of rabbit No. 8. We conjectured that the streptococcus would predispose the lung to infection with the streptothrix. This rabbit, killed 48 days later, was the only one of the four animals injected into the trachea that presented lesions.

Sections of the lungs reveal many pin-head to small pea-sized masses. The microscopical examination shows these masses to consist of intrabronchial new-formed tissue. The centres of many of these masses contain a homogeneous body, staining deeply with eosin, made up of the discoid granules of the streptothrix broth culture, which we shall refer to as the streptothrix clump. The peculiar morphological features of these clumps, which were present in the lesions of all the animals of the next series, are described under Morphology (p. 179).

The clumps have attracted around them a more or less dense zone of leukocytes, which is surrounded by a layer of necrotic tissue composed of ill-defined cells (staining deeply with eosin) with long and bizarre shaped nuclei or merely nuclear fragments. The fragmented nuclei of these tissue cells are arranged in lines of chemotactic attraction radiating towards the centre of the clump. The peripheral portions of the masses are formed by a spheroidal or polyhedral-celled tissue. When the intrabronchial mass of new tissue is of large size the normal bronchial structure is no longer seen, and the periphery of the mass is encapsulated by tissue of a more fibrous type. The peribronchial or alveolar walls adjacent to the masses are more or less extensively infiltrated by spheroidal cells.

In places areas of new tissue resembling granulation tissue occlude the bronchi and these may be so large that their original connection with bronchi cannot be definitely made out.

Besides the intrabronchial masses enclosing the streptothrix clumps there is a general catarrhal bronchitis and thickening of the bronchial coats by new tissue composed of spheroidal and polyhedral cells with here and there nodular projections of the submucosa into the lumen of the tube covered over by the bronchial epithelium. The lymphoid nodules of the bronchi are hyperplastic.

The first series of inoculations were in the main negative, the sole exception being rabbit No. 8, which alone of the four animals injected into the lungs, received, in addition to the streptothrix, a streptococcus culture.

The lesions in this rabbit consisted of catarrhal bronchitis with diffuse and nodular newly-formed connective tissue—productive bronchitis—hyperplastic lymphoid nodules, and the intrabronchial masses of tissue formed about the streptothrix clumps, or discoid granules of the broth culture. As will be seen from the description of the lesions of the rabbits of Series II, similar and even more marked lesions follow introduction into the lungs of larger quantities of streptothrix granules ob-

tained from broth cultures. The negative results of this series may therefore be attributed to the small quantities of streptothrix granules injected.

SERIES II.—In this series of inoculations larger quantities of streptothrix granules were injected. The granules were broken up as finely as possible so as to minimize their action as foreign bodies.

With the object of predisposing the lungs to infection with the streptothrix, after completion of the operation for tracheotomy, in all but one of the rabbits, a solution of 2 drops of concentrated ammonia in 15 drops of water was injected into the lungs through the trachea, followed 24 hours later by the intratracheal injection of the streptothrix culture. After each injection the small wound was stitched, and in none of the cases did infection of the wound follow. During the intratracheal injections the animals were inclined to the right side.

Rabbit No. 10.—April 3, intratracheal injection of ammonia followed 24 hours later by one-half of the streptothrix granules of a broth culture. Animal killed 37 days later. Pin-head sized foci and larger areas 5 to 6 mm. in diameter, surrounded by an œdematous translucent zone of tissue, are scattered throughout the lobes of both lungs. The lower left lobe is reddish in color and consolidated. Microscopical examination shows catarrhal bronchitis with considerable exudate in the larger tubes. The bronchial, peribronchial and alveolar walls are infiltrated with spheroidal and polyhedral cells. In the mucosa, nodular intrabronchial projections of new-formed tissue are found, which in places have caused a partial or complete obliteration of the lumen of the tube. The tissue resembles œdematous and vascular granulation tissue, and a drawing shows the canalization of the tissue with spaces lined by bronchial epithelium (Plate XV). In this rabbit there are areas of pulmonary atelectasis, and of lobular or broncho-pneumonia, the exudate consisting of leukocytes, epithelial cells, nuclear fragments and a few small giant cells. There is some necrosis of the cellular exudate. The alveolar walls are infiltrated by a few spheroidal or polyhedral cells. The adventitial coats and the intima of the larger blood-vessels are œdematous. Besides these lesions, the smaller bronchi are occluded by intrabronchial masses containing the streptothrix clumps, similar to those described in rabbit No. 8.

The pulmonary lesions in this rabbit, therefore, consist of intrabronchial masses of new tissue, enclosing the streptothrix clumps, of broncho-pneumonia—obliteration of some of the bronchial tubes and areas of lobular pneumonia with atelectasis.

Rabbit No. 11.—April 20, intratracheal injection of the streptothrix granules of a broth culture (15 days' growth), without the preliminary injection of ammonia. Animal killed 20 days after inoculation. About the trachea are found several large yellowish masses surrounded by fibrous tissue, due to escape of the culture through the track of the needle.

Scattered throughout the lungs small pin-head foci (Plate XII, Fig. 8) most numerous posteriorly, are found, similar in their microscopic appearances to the intrabronchial masses around the streptothrix clumps described above in rabbits Nos. 8 and 10. No other lesions are present in the lung.

Rabbit No. 12.—April 4, intratracheal injection of ammonia followed 24 hours later by an intratracheal injection of half of the streptothrix granules of a broth culture (15 days' growth). Slight embarrassment to respiration followed both injections; animal killed 36 days later.

Small (5 to 6 mm.) flat masses surrounded by a translucent zone project beneath the pleura. The posterior portion of the lung, reddish in color, is consolidated by areas of lobular pneumonia. The intrabronchial masses around the streptothrix clumps are larger than those of the preceding rabbits. The largest masses situated beneath the pleura, which is congested, œdematous and thickened, are composed of a large central collection of pus cells and necrotic tissue, with an occasional streptothrix clump and are encapsulated by an extensive zone of well-formed spheroidal or polyhedral-celled tissue, which extends diffusely beneath the pleura into the adjacent lung tissue. A few arteries present an obliterating endarteritis. The lesions, judging from the larger size of the abscesses, are more severe than those of rabbit No. 10. The encapsulation by fibrous tissue testifies to the successful conservative attempt on the part of the lung to limit the process.

Rabbit No. 13.—May 25, intratracheal injection of ammonia followed 24 hours later by injection of large quantities of streptothrix granules obtained from various broth cultures. Twelve days later the animal was killed.

At autopsy there are several large yellowish masses resembling inspissated pus, surrounded by a fibrous capsule in the peritracheal tissue due to escape of the injected fluid. There is a sero-fibrinous mediastinitis. The upper lobe of the right lung contains reddish, collapsed areas, and yellowish-white pea-sized masses project beneath the pleura. White pin-head sized masses project beneath the pleura. White pin-head sized foci are scattered throughout the lobes of the lungs.

The streptothrix was recovered in pure culture from the peritracheal and from one of the pleural masses in the upper lobe.

Microscopically the subpleural masses resemble those of rabbit No. 12. The foci consist of alveoli filled with spheroidal and epithelial cells. The right upper lobe is extensively atelectatic, and the alveoli contain exfoliated epithelium and large multinucleated giant cells. There are also areas of interstitial tissue of the ordinary type.

Rabbit No. 14.—May 25, intratracheal injection of ammonia followed 24 hours later by injection of large quantities of streptothrix granules, obtained from the same broth cultures with which rabbit No. 13 was injected. Animal, much emaciated and with marked dyspnœa, was killed 12 days after inoculation.

The autopsy showed catarrhal bronchitis with much exudate. A large cavity with dense fibrous walls and filled with creamy semi-solid material occupies nearly the whole upper lobe of right lung. Numerous smaller cavities and white foci are scattered throughout the various lobes of both lungs. The pericardium and left lung are adherent to the chest wall and there is sero-purulent pericarditis.

Microscopically the cavities and abscesses are seen to be surrounded by extensive zones of œdematous granulation tissue. The lung tissue between the abscesses is atelectatic or consists of new-formed fibrous tissue. The pus in the cavity and abscesses contains numerous rods and cocci and a few streptothrix clumps. The necrotic walls of the cavity likewise contain cocci and rods. Cultures yield a variety of microorganisms which were not identified.

The lungs resemble those of the rabbits which were injected through the trachea with the streptothrix masses and pus obtained directly from the original human cases. The result in this experiment indicates that cavity formation and severer lesions are produced when there is concurrent infection with cocci. In this case the cocci were either introduced accidentally at the time of injection, or entered later from the air passages.

Rabbit No. 15.—May 12, intratracheal injection of ammonia followed 24 hours later by injection of the streptothrix granules obtained from various broth cultures. Animal killed 10 days later. Near the trachea two yellowish almond-sized lumps encapsulated by radially striated dense fibrous tissue are found. The lumps are due to the escape of the fluid at time of injection through the perforation in the wall of the trachea.

Upper lobe of right lung is dark red in color, consolidated, somewhat collapsed, and does not distend when the lungs are injected. Sev-

eral confluent whitish dime-sized nodules are seen beneath the pleura. Scattered throughout all the lobes a few subpleural pin-head sized whitish nodules are found.

Microscopically the consolidated lobe is seen to be atelectatic. The subpleural nodules are abscesses which contain a few streptothrix clumps and resemble those of rabbit No. 11. The pleura over the abscess is thickened by a tissue rich in spheroidal cells and leukocytes.

Smears made from masses around the trachea reveal a few rods with bipolar staining, and of the two broth tubes planted with large loopfuls of the material, one alone yields a delayed growth of the streptothrix.

The sections of the abscesses show a few streptothrix clumps without isolated rods. Other forms of bacteria were not seen. The remaining lobes contain smaller intrabronchial masses which surround the clumps, and there is a general catarrhal and productive bronchitis with nodular projections into and obliteration of the lumina of the tubes. The adventitia of the small blood-vessel is frequently infiltrated with spheroidal cells. The vascular changes otherwise resemble those described above.

SERIES III.—The following inoculations are for convenience grouped together.

Intraperitoneal injection: Rabbit No. 16.—April 20, injected into the peritoneum numerous granules of a streptothrix broth culture (15 days' growth). Rabbit killed 25 days later. Over site of injection, attached by a pedicle, is a pea-sized mass, hanging freely in the peritoneal cavity. Six small yellowish nodules adhere to the mesentery of the stomach and to the liver. The mesenteric lymph nodes are not affected. A yellowish-white, small, almond-sized mass is attached to the cæcum by fresh adhesions. On section the larger masses are made up of several confluent nodules, each surrounded by dense fibrous tissue. Smears made from the material reveal a few bipolar-staining rods. Small bits of the material were planted in four broth tubes, of which two after two weeks at 37° C. develop the clumpy variety of streptothrix culture.

The yellowish nodules forming the pendulous mass consist of a central collection of fragmented faintly-staining nuclei and necrotic granular material with a few clumps and clusters of streptothrix surrounded by a zone of vascular connective tissue, composed of spheroidal and larger polyhedral cells and fibroblasts, with eosinophilic granulations. The nodules on the serosa of the liver are of similar structure, the parenchyma of the liver being unaffected. Some of the nodules, however, are formed exclusively of vascular granulation tissue, and represent the nearly healed lesions. (Compare the result in this case with that in rabbits Nos. 1 and 2, in which the process of healing is complete, p. 181.)

Subcutaneous injection: Rabbit No. 17.—Feb. 25, injected subcutaneously the granules of an early broth culture. Twenty-four days later a small almond-sized nodule formed near the site of injection was removed. Coverslips revealed no organisms and no cultures were made. The mass on cross section is yellowish in color, resembles inspissated pus and is encapsulated, with a radially striated border of fibrous tissue.

In the sections stained by Weigert and by Sterling's gentian violet, a few clusters of streptothrix are found. The rods are similar to those met in the sections of the human lungs. The central part of the mass consists of a collection of leukocytes and necrotic material surrounded by vascular granulation tissue, which becomes densely fibrous at the periphery.

Intraperitoneal inoculations into guinea-pigs.—Three guinea-pigs were injected with large quantities of streptothrix culture obtained from various sugar-broth tubes on May 13.

The smallest animal became emaciated and died 13 days later. A few pin-head sized whitish nodules were found loosely attached to the mesentery. Smears reveal a few rods with polar staining; the inguinal lymph nodes were slightly swollen. The second animal was killed two weeks after inoculation. Negative autopsy. The third died 18 days after inoculation. Marked emaciation. A few small white nodules in the peritoneum similar to those found in the first guinea-pig. No cultures were made. No conclusions can be drawn from these inoculations in guinea-pigs, as they were not repeated.

Summary of the Experimental Lesions Produced by Inoculations of Pure Cultures of Streptothrix.—The streptothrix is pathogenic to rabbits and guinea-pigs, but death rarely supervenes from its action. In the rabbit subcutaneous injections and the escape of broth cultures into the peritracheal tissues, and intraperitoneal injections in rabbits and guinea-pigs produce local death of tissue and abscesses encapsulated by extensive zones of granulation tissue. In the lesions a few clusters of well-stained filaments or rods may occasionally remain, but individual rods are rarely found. The few clumps which are present in all the lesions represent, we believe, the discoid granules of the broth culture, which have undergone a peculiar transformation.

Introduction of pure cultures into the lungs through the trachea

is followed by similar phases of reaction on the part of the blood-vessels and of the tissue cells. When large quantities of the finely broken streptothrix granules of broth cultures are injected into the lungs, areas of consolidation composed of proliferated epithelial cells and leukocytes are formed, in addition to the intrabronchial masses of new-formed tissue, which encapsulate the clumps or discoid granules of the cultures. The largest intrabronchial masses in their early stages (two weeks after inoculation) resemble abscesses.

RESUMÉ.

The two cases of broncho-pneumonia in man forming the subject of this article were characterized by intense catarrhal and necrotic inflammation of the bronchi and by the presence of numerous streptothrix colonies in the bronchial lumina.

Introduction of the bronchial material of Case I into the trachea of three rabbits induced pulmonary abscesses and empyema of the pleura and pericardium in one of the animals.

Introduction of the bronchial material of Case II into the ear vein of a rabbit, and into the trachea of a second rabbit induced likewise pulmonary abscesses and empyema.

The empyemal pus of these rabbits contained filaments and rods morphologically identical with those composing the streptothrix colonies of the human cases.

From Cases I and II a streptococcus was cultivated on the ordinary media, the streptothrix not being isolated in culture directly from the human organs. By inoculating the fresh and sterile kidneys removed from a normal rabbit with the empyemal pus of a rabbit injected into the ear veins with the bronchial material of Case II the streptothrix from this case was finally isolated in pure culture, and its morphological and biological characters studied in detail.

COMPARISON WITH SPECIES PREVIOUSLY REPORTED.—Space does not permit a review of the records of the numerous species of the genus Streptothrix which have been found to bear a causal relation to various lesions or diseases, nor can we dwell upon the interesting rela-

tionships of the Streptothrices to a number of microorganisms which heretofore have been considered bacilli or fissure fungi.

For many years Actinomyces bovis Harz (Streptothrix actinomyces Rossi Doria) was considered to be the only representative of a genus of microorganisms to which the name "streptothrix" is at present generally applied.[1] The growth flourished best under aërobic conditions and consisted of a felted network of branching mycelia mixed with shorter coccus- or spore-like forms. Inoculations of the cultures in animals were regularly negative.

Wolff and Israel in 1889 cultivated from two typical cases of actinomycosis a different species, Streptothrix Israeli, which flourished best under anaërobic conditions. In contrast to the morphology of the aërobic species the growth consisted mainly of rods with a few branching filaments, except in egg cultures where branching masses of mycelia developed. It grew only at incubator temperature, whereas the aërobic species developed equally well at room temperature. Typical actinomycotic lesions with rosettes and terminal bulbs followed introduction of the cultures into the peritoneum of rabbits.

Wolff and Israel's observations were not confirmed until A. Aschoff in 1895 cultivated a species similar to Streptothrix Israeli from a case of pulmonary actinomycosis.

Recently E. Levy has reported five cases of human actinomycosis from which he cultivated a strictly anaërobic species, which closely resembled Streptothrix Israeli.

Actinomyces bovis and Streptothrix Israeli are now considered to be two distinct species of streptothrix. The failure of Lucas, working in Levy's laboratory, to transform the aërobic species (Streptothrix actinomyces) into the anaërobic variety, with the morphological characteristics of Streptothrix Israeli, by cultivation through many generations under anaërobic conditions of growth, speaks against the identity of the two microorganisms. Levy does not favor the view held by some that Streptothrix Israeli is a pleomorphic bacillus.

[1] Inasmuch as the name "streptothrix" had been previously appropriated for one of the hyphomycetes by Corda in 1839, it has been proposed by Levy to designate the group now generally called "streptothrix" as "actinomyces." We have, however, followed in this article the more usual designation at present.

Hugo Bruns has isolated what he considers to be a transitional form between the two species. In its cultural and morphological characteristics it closely resembles Streptothrix Israeli, but differs from it, in that it grows best aërobically.

The species isolated by us resembles closely in its morphological and cultural characteristics Streptothrix Israeli; but it is decidedly less pathogenic to animals. We do not consider the anaërobic preferences of Streptothrix Israeli sufficiently marked to form a point of differentiation between it and the species isolated by us, or by Bruns, since Wolff and Israel mention successful primary cultivation of their streptothrix from their first case on slant agar.

On account of the resemblance of Bruns's agar cultures to those of the tubercle bacillus it cannot be identified with ours, and the same objection presents itself in regard to the streptothrix isolated by Foulerton which he considers resembles the species isolated by Bruns.

Berestneff has isolated five species of streptothrix from human cases and although one of his species resembles ours in regard to its morphology and its broth cultures, we do not consider his descriptions sufficiently complete for purposes of species identification.

Paul Kruse cultivated from the yellowish granules in the pus of a maxillary tumor in a diabetic barber, a streptothrix which he considers closely related to or identical with Streptothrix Israeli. Except for the formation of acid in milk and broth cultures our species does not differ from Kruse's as far as one can judge from his descriptions.

Our streptothrix thus would seem to be, if not identical with, at least closely related to Streptothrix Israeli, and to the species isolated by Kruse. Whether it can be considered a variety or transitional form of Levy's anaërobic species must be left undecided.

The pulmonary lesions of the cases, however, do not resemble the broncho-pneumonic type of pulmonary actinomycosis described in the literature. The intense necrotic inflammation of the bronchi of the two cases reported, distinguishes them from the heretofore recorded cases of pulmonary actinomycosis or pseudo-actinomycosis.

We desire to express our thanks to Dr. A. Seibert and to Professor Delafield for the privilege of reporting these cases, and to Dr. Eugene Hodenpyl, Pathologist to the Roosevelt Hospital, for the opportunity of studying Case II.

It gives us great pleasure to acknowledge our deep sense of obligation to Prof. T. Mitchell Prudden for the kind assistance and suggestions which have always been at our disposal.

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DESCRIPTION OF PLATES XI-XVI.

PLATE XI.

Fig. 1.—Rod-like forms of streptothrix of Case II, from 2 days' growth of 3d generation on rabbit's kidney. Gram's stain.

Fig. 2.—Smear from colony, 7 days' growth, on glycerin agar, stained with methylene blue. Shows beaded filaments, small rods, and coccus-like forms. Compare with Fig. 3.

Fig. 3.—Smear from same colony as Fig. 2, stained by Gram. Note the differences in morphological appearances brought out by the two stains used for Fig. 2 and Fig. 3.

Fig. 4.—Coverslip from 3 days' growth in sugar broth. P. Ernst's stain. Shows branching forms and deeply stained granules.

Fig. 5.—Photograph showing streptothrix colonies growing on rabbit's kidney.

PLATE XII.

Fig. 6.—Photograph of section of rabbit's lung (Rabbit 3806) showing pneumonia and cavity produced by streptothrix infection following intraperitoneal inoculation of empyemal pus of infected rabbit.

Fig. 7.—Areas of infarction and abscesses in lung of the same rabbit (Rabbit 3806).

Fig. 8.—Scattered foci of consolidation in lung of rabbit killed 20 days after intratracheal inoculation with broth culture of streptothrix (Rabbit No. 11). With higher power these foci show intrabronchial masses of connective tissue growing around streptothrix clumps.

PLATE XIII.

A. Rods, filaments and curious forms of streptothrix seen in sections of bronchi in the human cases. They are stained reddish brown by Gram's method, this reaction being due to iodine.

B. Streptothrix forms from 10 days' growth of 14th generation in bouillon stained red in hot carbolic fuchsin, the intense color being retained after decolorization of the specimen in 97% alcohol.

PLATE XIV.

Streptothrix colony surrounded by cells and organizing tissue in rabbit's lung. The bulbous ends of the filaments are deeply stained by Weigert's fibrin dye.

PLATE XV.

Obliteration of rabbit's bronchus by new growth of connective tissue, the plug being channeled with canals lined by bronchial epithelium. The new tissue appears œdematous. The section is from the lung of Rabbit No. 10, killed 37 days after intratracheal injection of broth culture of streptothrix (p. 183).

PLATE XVI.

Section of lung of Case I showing necrotic and suppurative bronchitis, with streptothrix clumps within the necrotic material in a bronchus. Fibrin and cells in the surrounding alveoli.

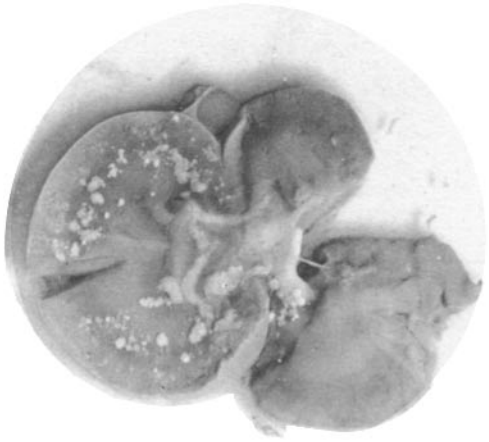


FIG. 5.

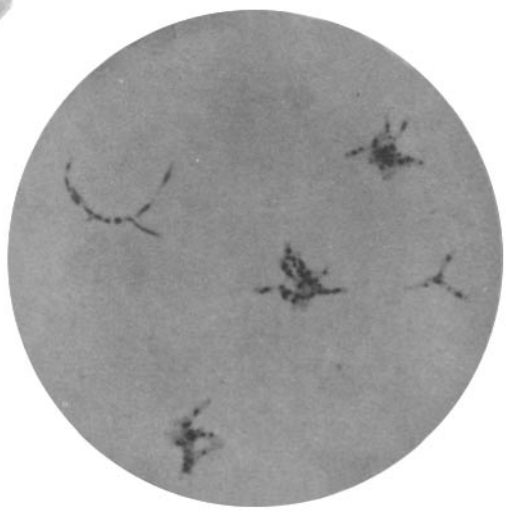


FIG. 4.

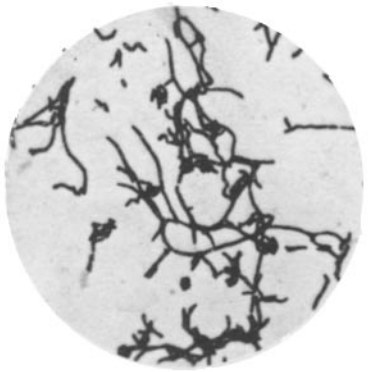


FIG. 3.

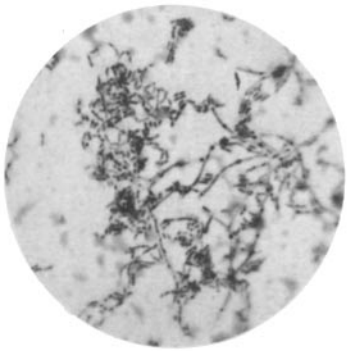


FIG. 2.

FIG. 1.

THE HELIOTYPE PRINTING CO., BOSTON.

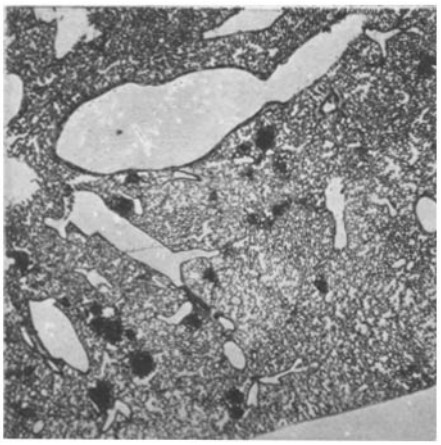


FIG. 8.

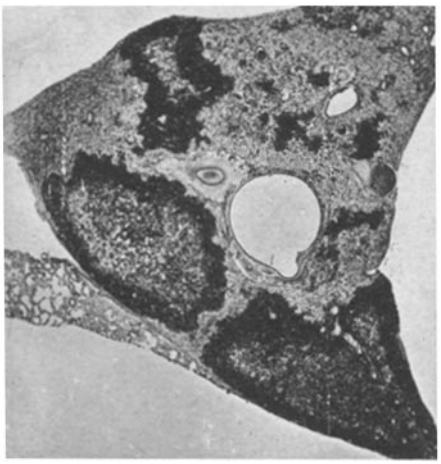


FIG. 7.

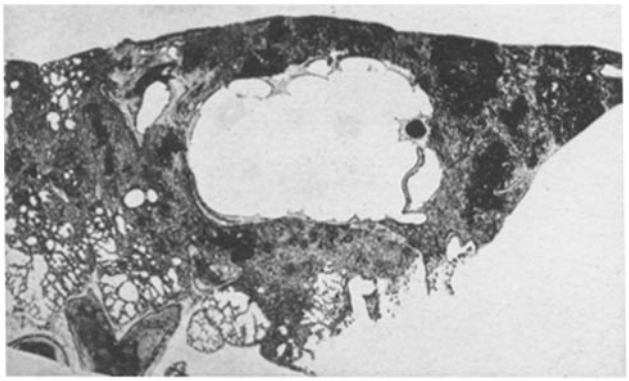
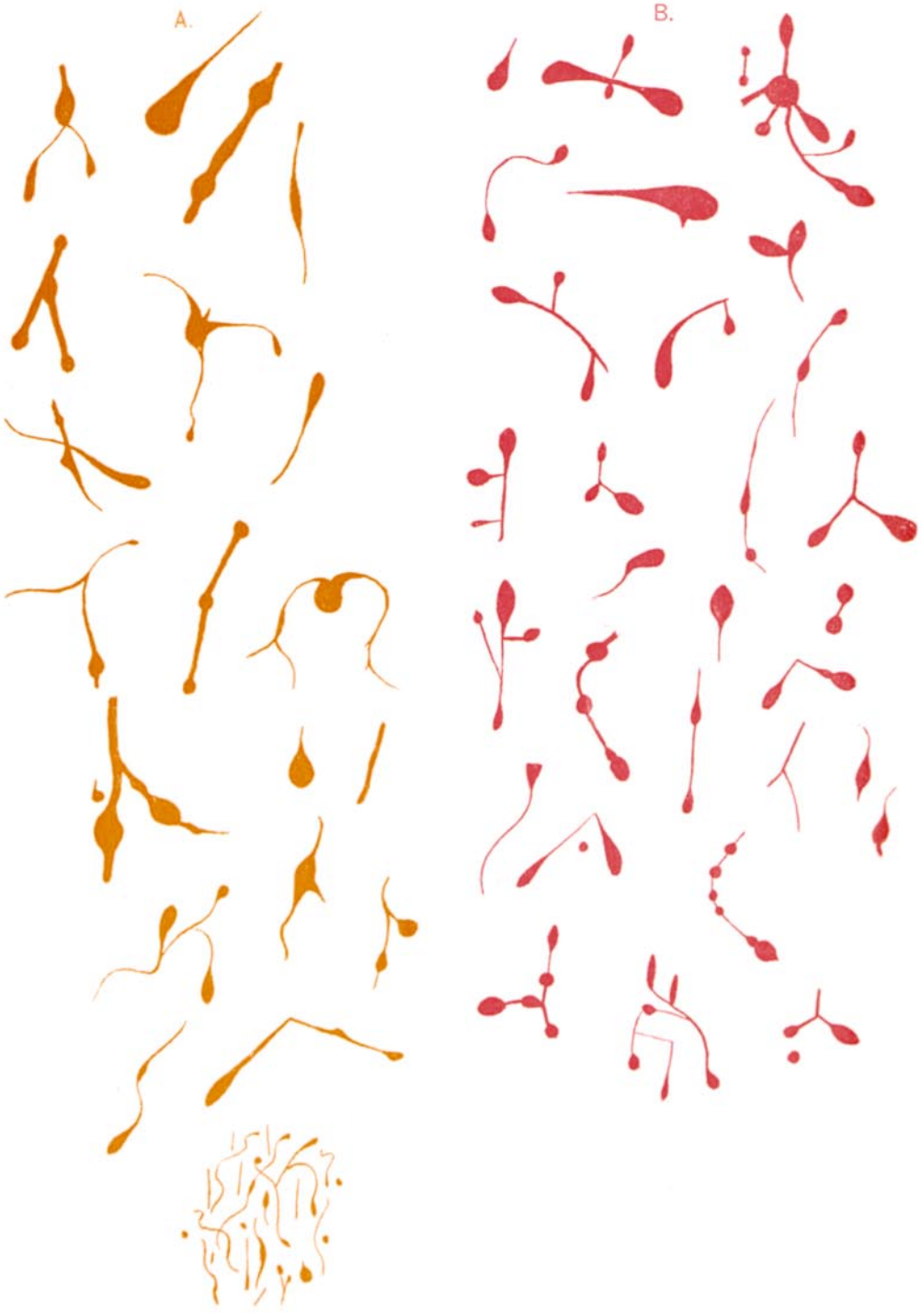


FIG. 6.

THE HELIOTYPE PRINTING CO., BOSTON.


A.
B.