ACTINOBACILLOSIS OF MAN *

REPORT OF A FATAL CASE

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In 1902 Lignières and Spitz,[1] working in the Argentine, identified the *Actinobacillus* as one of the etiological agents of so-called bovine actinomycosis. They proposed the designation "Actinophytose à actinobacille" for the disease, which by subsequent investigators has been called actinobacillosis.

Clinically and pathologically bovine actinomycosis and actinobacillosis have few distinguishing characteristics. The chief point of difference is situation. Actinomycosis usually involves the jaw bones as a rarefying osteitis with formation of sinuses, whereas actinobacillosis more often attacks the soft tissues of the mouth, pharynx, tongue and skin, with secondary involvement of the adjacent lymph nodes. Notwithstanding the clinical and pathological similarities of the diseases, the causal organisms are quite distinct, except in one feature. Both have the property of evolving aggregations in the tissues and exudates known as sulphur granules, about which radiating clubs are easily identified. On crushing the granules, however, the *Actinobacillus* yields only small Gram-negative bacillary forms, whereas *Actinomyces* reveals the distinctive branched Gram-positive filaments. Lignières and Spitz found the *Actinobacillus* capable of producing an experimental disease of cattle identical with the natural infection. It possessed a variable virulence for guinea pigs and rabbits.

The conclusions of Lignières and Spitz have been confirmed repeatedly by investigators working on the continent of Europe, the British Isles and Canada. Recently Thompson [2,3] reviewed the subject, and reported for the first time the existence of actinobacillosis as a common malady of cattle in the United States. He concluded: "Actinobacillosis is common among cattle in the United States. It would seem that the condition here is similar to that in other coun-

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tries where the greater percentage of so-called bovine actinomycosis is due to *Actinobacillus lignièresi*." For more complete consideration of the subject reference may be made to Thompson's paper.

Thompson and Willius [4] in 1932 reported the identification of *Actinobacillus* bacteremia in man. The patient was subsequently observed by Lawrence, Neuhauser and Howell [5] and in a confirmatory, supplementary report it was stated by these observers that the illness had apparently terminated in recovery. Ravaut and Pinoy [6] in 1911 described the occurrence of *Actinobacillus* meningitis. Their patient likewise recovered. The present case of *Actinobacillus* infection, the third in man to be reported, was fatal; consequently it is the first in which pathological studies have been made. The presentation of this case is our objective in this paper.

Report of Case

Clinical History: A man, aged 34 years, came to The Mayo Clinic Jan. 9, 1933. He had been employed as a clerk in a clothing store until a few months before, and more recently had been engaged in selling floor wax throughout southern Minnesota. He was born in southeastern Minnesota, where he always had resided. His health had been quite normal until the summer of 1932, when he began to lose weight gradually, for no apparent reason. Periods of anorexia were sometimes experienced. All bodily functions apparently had remained normal. He had not experienced excessive thirst or polyuria. There was no cough. On Dec. 25, 1932, he had no appetite, felt poorly and that evening retired early. On December 27 he became acutely ill and went to bed. The illness was considered by the patient to be influenza; it was characterized by malaise, weakness, fever to 102° F, and profuse perspiration. About Jan. 1, 1933, severe pain developed in the thorax posteriorly between the shoulders. Plasters were applied and on January 7 the pain entirely disappeared. The illness, however, was progressive; appetite failed, weight was lost more rapidly and weakness increased to the point of exhaustion. Fever continued, and on January 8 drowsiness and mild delirium supervened.

On the patient's admission to the clinic he appeared to be acutely ill and very weak. His temperature was 102° F, pulse 116 beats and respiration 32. Rather rapid loss of weight was evident (from 185 to 135 pounds). The skin was warm and moist. The eyes were rather prominent. The tongue was coated and the pharynx hyperemic. An enlarged cervical lymph node was palpable on the right side. A friction rub was noted in the right midclavicular line at the level of the fourth rib. Resonance was impaired at the bases of both lungs and moist râles were elicited at the base of the left lung. The heart was slightly enlarged, by percussion. The abdomen was distended and held tense; peristalsis was active. Roentgenograms of the thorax were interpreted as revealing extensive pneumonic consolidation at the base of the left lung, probably with fluid. A moderate degree of anemia was present. The concentration of hemoglobin was 69 per cent, erythrocytes numbered 3,510,000 and leukocytes 7000 in each cmm. of blood. The percentages of the various types of leukocytes were as

follows: polymorphonuclear neutrophils 77, lymphocytes 12 and monocytes 11, with changes in the leukocytes indicative of severe infection. Urinalysis revealed albumin, graded 2; sugar, graded 2; hyaline casts, graded 4, and an occasional erythrocyte; acetone was not present. Urea was present in the blood in essential normal concentration (44 mg. in each 100 cc. of blood), and sugar elevated (326 mg. in each 100 cc. of blood). Agglutination reactions for typhoid, paratyphoid and undulant fevers were negative. The Wassermann reaction of the blood was negative.

The patient was placed on a diabetic regimen and with use of insulin the blood sugar was brought to within normal limits. The fever increased to 103° F, and remained elevated, except for a rather sharp fall to 98° F on the morning of January 11, with subsequent rise to 102.8° F by noon of that day. The pulse and respirations remained rapid. On the evening of January 11 his breathing became more labored and respirations markedly accelerated. He was placed in an oxygen tent but failure was progressive and rapid. Death occurred at 12.17 A.M., Jan. 12, 1933.

At the time of death the diagnosis as to type of infection still was indeterminate. In many respects the illness resembled typhoid fever, and also influenzal pneumonia. The pneumonia, however, was atypical. The severity of the illness seemed to be out of all proportion to the physical or laboratory findings. The evidence of severe toxemia did not appear to be explained by the findings noted in the thorax, or by the diabetic state. Cultures of blood, which had been taken January 11, still revealed no growth of microörganisms when death occurred. The onset of symptoms of the fatal infection was probably about Dec. 25, 1932, and the preceding loss of weight was the result of diabetes. It is also probable that the presence of diabetes accentuated the infection and accounted for its severity, with rapidly fatal termination.

Pathological Data

Throughout both lungs were multiple, firm, grayish nodules that measured from 2 to 5 mm. in diameter. The nodules were subpleural and were also within the substance of the lung. Nodules observed beneath the pleura were distinctly circumscribed and somewhat elevated. From some of them thick, yellowish pus exuded on section, whereas others were composed of grayish consolidation or caseous material. The spleen weighed 748 gm.; it was embedded in a mass of adhesions, and was composed largely of multiple, discrete, sharply circumscribed, grayish yellow lesions varying in size from 1 mm. to 1 cm. (Fig. 1). On section these lesions contained thick yellow pus or material of thick pasty consistence, which resembled

that of caseation necrosis. The liver weighed 2600 gm. The hepatic lesions were similar in appearance to those observed in the spleen; however, they were less numerous and somewhat smaller. The substance that composed these foci resembled caseation necrosis rather than pus. One very small abscess was observed in the cortex of the left kidney. Other organs appeared to be normal. The brain and spinal cord were not examined.

Microscopic Examination: Fresh tissue was fixed in Orth's fluid, embedded in paraffin and stained with hematoxylin and eosin. The lesions are essentially the same in all anatomical situations, as observed in sections of the lungs (Fig. 2), liver (Fig. 3), spleen and left kidney. They consist of focal, sharply circumscribed zones of exudation with usually advanced necrosis of the exudate centrally and granulomatous proliferative changes peripherally. Early lesions are composed almost entirely of polymorphonuclear neutrophilic leukocytes without necrosis or peripheral granulomatous reaction. The usual and typical lesion, however, consists of the three zones: (1) a central zone of necrosis composed of granular, eosin-staining acellular material in which chromatin dust-like fragments of cell nuclei persist; (2) a wide zone about the central zone of necrosis composed of polymorphonuclear neutrophilic leukocytes in which a few lymphocytes and large phagocytic mononuclear leukocytes are also found; and (3) a peripheral zone about each lesion consisting of gradually diminishing numbers of leukocytes and gradually increasing numbers of endothelioid cells, fibroblasts, and occasionally also young blood vessels. The latter area varies from lesion to lesion, so that in some it comprises only a small portion, while in others it represents a well defined zone. The three zones are not especially distinct from one another, but each appears to blend gradually with the adjacent one. No further encapsulation exists, but adjacent to each lesion there is atrophy of parenchymal cells together with retrogressive cellular changes. Around some of the pulmonary lesions, however, there is an organizing pneumonic process for a considerable distance. In the peripheral portions of the lesions large compact aggregations of bacteria in colony formation are numerous. These masses are composed of faintly stained, small Gram-negative bacillary forms with a tendency to bipolar staining. Single organisms in the tissue are difficult to find, but a few are seen as plump, Gram-negative rods. They are not acid-fast.

Bacteriological Data

In a culture from the blood, made 24 hours before death, Gram-negative, rod-shaped forms appeared in the broth after 2 days. At the same time, a few colonies of similar organisms appeared in the agar plates. There was less than one colony for each cc. of blood used. At autopsy cultures were obtained from the blood, spleen, liver and lung. From all of these sources organisms were obtained having the following characteristics: Medium sized, Gram-negative, non-motile rods, occurring singly or in short chains in broth cultures. On blood agar, after 24 to 48 hours, the colonies were 1 to 2 mm. in diameter, round, convex and milky white. There was no effect on the blood. On nutrient agar the colonies were similar but smaller. In nutrient broth a pellicle and sediment were formed. Ehrlich's test for indol was positive after 3 days. On potato slants there was no visible growth after 6 days. Blood serum and gelatin were not liquefied after 15 days. On glycerin agar the growth was white, shiny, flat and membranous after 5 days. In sugar fermentations acid but no gas was produced in glucose, maltose, mannite and salicin. Neither acid nor gas was produced in lactose, saccharose, dulcitol and inulin. The cultural characteristics are summarized in Table I, in which the organism isolated from the present case is designated by the letter Z. For comparison the table includes the strain of *Actinobacillus* previously isolated from a human being, and in addition, typical strains of *Bacillus whitmori*, *Pfeifferella mallei* and *Actinobacillus* of bovine origin. Thompson [7] has recently recommended that these organisms be included in the genus *Actinobacillus*.

The serological relationship of the organism was determined by immunizing rabbits to produce an agglutinating serum of high titer. Similar serums were prepared with a culture of *Actinobacillus* of bovine origin and a culture of *Pfeifferella mallei*. The results of cross agglutination tests are shown in Table II. Serum Z is the one prepared with the organism isolated from the present case. Table II also includes, for comparison, the results obtained with an organism from a previous case and with a culture of *Bacillus whitmori*. Attention is called to the fact that all of these serums give a positive complement fixation test with an antigen prepared from *Pfeifferella mallei*. Although it does not appear in the table, tests were made

TABLE I

Cultural Characteristics of Genus Actinobacillus

Culture	Motility	Glycerine agar	Gelatine	Blood serum	Potato	Indol	Sugar fermentation							
							Glucose	Maltose	Mannite	Lactose	Saccharose	Dulcitol	Inulin	Salicin
1*	–	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	A**	A	A	A	A	o	o	o
99†	+	Wrinkled, yellow	Liquefied	Liquefied	Growth good, yellow	–	A	o	Sl. A	Sl. A	o	o	o	o
PD‡	–	Smooth, yellow	Not liquefied	Not liquefied	Growth good, yellow	Sl. +	o	o	o	o	o	o	o	o
M§	–	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	Sl. A	o	Sl. A	o	Sl. A	o	o	o
Z§	–	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	A	A	A	o	o	o	o	A

* 1 = *Actinobacillus* (bovine)
** A = Acid
† 99 = *Bacillus whitmori*
‡ PD = *Pfeifferella mallei*
§ M and Z = *Actinobacillus* (human strains)

which showed that serum Z did not agglutinate *Alcaligenes abortus* and *Pasteurella tularensis*.

To summarize the information contained in the tables it may be said that the organism under consideration shows a group relationship with *Pfeifferella mallei* and with *Bacillus whitmori*. The latter organism is the cause of a glanders-like disease in southeastern Asia called melioidosis. It also resembles more strongly *Actinobacillus*

TABLE II

Serological Reactions of Actinobacillus

Cultures	Rabbit serums prepared with culture					Complement fixation with mallei antigen
	1	99	PD	M	Z	
1*	10,240	0	20	40	160	44400
99†	0	2560	40	0	0	44444
PD‡	20	2560	2560	20	20	44444
M§	0	80	0	320		44400
Z§	80		80		5120	44400

* 1 = *Actinobacillus* (bovine)
† 99 = *Bacillus whitmori*
‡ PD = *Pfeifferella mallei*
§ M and Z = *Actinobacillus* (human strains)

lignièresi, the cause of bovine actinobacillosis, but differs from the latter in certain sugar fermentations. From the study of two strains obtained from human beings it seems most logical to consider these organisms as *Actinobacillus lignièresi*, which show slight cultural and antigenic differences from the strains of bovine origin.

It is possible that infections due to *Actinobacillus lignièresi* have not been recognized in the past, owing to the superficial similarity of this organism to those of the coli group. All Gram-negative, nonmotile, aerobic organisms that produce only acid in sugar mediums should be investigated more fully as to cultural and serological reactions.

Experimental Data

Triturated preparations obtained from the spleen, liver and lung of the patient at autopsy, ante mortem blood, and pure cultures as recovered from the ante mortem blood culture and from the lesions at autopsy, were proved to be pathogenic for rabbits, guinea pigs and mice. In these animals granulomatous abscesses and tubercle-like granulomas were evolved. The granulomatous features in the lesions were frequently much more prominent than they were in the lesions of human beings; nevertheless, they were similar if not identical with one another. A clearly evident Strauss reaction was never elicited, although in two guinea pigs mild periorchitis was revealed at autopsy. Bacterial masses in ball-like colony formation were found in many of the infectious foci of the experimental animals, similar in appearance to those that were seen in the lesions of human beings. Well defined peripheral clubs were not associated with these colony formations, as in bovine actinobacillosis, although suggestions of early club formations were sometimes observed. In coverslip preparations of the exudates the organisms appeared usually as single, plump, faintly stained Gram-negative rods, sometimes in pairs or in short filaments, many revealing bipolar staining characteristics.

The lesions in guinea pigs and rabbits also, in a general way, resembled those described for experimental glanders. The experimental disease produced by the organism from our case was chiefly distinguished from experimental glanders by failure to produce a characteristic Strauss reaction in guinea pigs, the colonization of the bacteria in aggregations in the lesions and by revealing greater pathogenicity for rabbits than for guinea pigs.

The strain of *Actinobacillus* from our case was of greater pathogenicity than generally has been described for the *Actinobacillus* of bovine origin. Lignières and Spitz [8, 9] found that their cultures had variable degrees of virulence for bovines as well as for small laboratory animals, although they distinctly described the evolution of fatal peritonitis on intraperitoneal inoculation of guinea pigs, with death of the pigs in from 12 to 24 hours when large doses were used, and in from 5 to 7 days when smaller doses were used. The character of the peritonitis produced in their animals was apparently comparable with that revealed by ours. The suggestion of a mild Strauss reaction in two of our animals is also in accord with their description

of periorchitis, which developed in guinea pigs inoculated intraperitoneally, although this reaction in their animals was of much greater severity. In the lesions of guinea pigs inoculated intraperitoneally Lignières and Spitz found the tufts or aggregations of microörganisms with peripheral clubs similar, although less distinct, to those evolved in bovine lesions. In our experimental guinea pigs, especially those inoculated intraperitoneally, the aggregations of bacteria in compact colony formation were very prominent in many of the lesions, although the zone of peripheral clubs was either only faintly suggestive or not revealed. Neither Magnusson [10] nor Griffith [11] was able to confirm this observation of Lignières and Spitz. The formation of subcutaneous abscesses in guinea pigs, which developed on subcutaneous introduction of the organisms such as we describe, was also produced by bovine strains in the experiments of other investigators.

Rabbits inoculated with the strain from our case were more readily infected than other observers have found them to be when working with bovine strains of the organism. There is perhaps no record of the production of lesions by the *Actinobacillus* in rabbits comparable to those exhibited by our animals. It is therefore apparent that in this instance we have a strain of virulence of high degree, which may account in part for the fulminating infection of our patient and also for the ease with which lesions were evolved in animals. In the case of *Actinobacillus* bacteremia studied by Thompson and Willius the organism was much less virulent for both man and laboratory animals. This is what one would usually expect, judging from the experience of other observers in working with bovine strains.

Rabbits: These animals appeared to be most susceptible to the infection (Table III). In Rabbit 1, which received an intravenous inoculation of 1 cc. of a 24 hour pure brain broth culture, the greatest virulence of the organism was manifest, death of the animal resulting in 29 hours. At autopsy small, scarcely visible, granulomatous foci were found in the liver, lungs and spleen. Microscopically these consist of focal areas of necrosis with early polymorphonuclear leukocytic exudation and beginning proliferation of endothelioid cells. The lesions are discrete, multiple and particularly prominent in the liver and spleen. Rabbits 2, 3 and 4, which received 0.3, 0.8 and 1.5 cc. of a pure 24 hour brain broth culture intravenously, lived 15, 11 and 5 days respectively. Clinically, all of these rabbits revealed

a slowly developing but progressive general reaction in which loss of appetite, emaciation and listlessness were the chief symptoms. At autopsy lesions were found similar to those seen in Rabbit 1, but more advanced. In each of the animals lesions were observed in the liver, lungs and spleen. In Rabbits 2 and 3 the kidneys also were involved, and in Rabbit 2 the cecum. In Rabbits 2 and 3 the lesions were particularly well defined. Grossly in each instance the spleens were enlarged to approximately five times normal size and were mottled by grayish areas of necrosis, sometimes discrete, but due to the enormous number of lesions becoming confluent (Fig. 4). In the livers and lungs the lesions were extremely small and appeared as a fine grayish white mottling. In the kidneys and cecum larger grayish necrotic foci appeared. Microscopically there is considerable variation in the appearance of the lesions, but they are still compatible with stages of the same granulomatous process. Briefly stated, the lesions consist of a central zone of necrosis in which nuclear detritus is rather prominent. Surrounding this polymorphonuclear leukocytes have collected and peripherally a rather well defined zone of endothelioid cells is observed. In some instances the outer portion of this area is composed mostly of fibroblasts. Interspersed with the endothelioid cells and fibroblasts are a few polymorphonuclear leukocytes, lymphocytes, mononuclear leukocytes and plasma cells. The zone of necrosis varies so that in some instances it occupies almost completely the entire focus with just a narrow rim of cellular elements, whereas in other lesions it is extremely small or almost absent. When the latter prevails the center of the lesion is either occupied by polymorphonuclear leukocytes as the predominating cell, or the entire structure is evolved as a tubercle, consisting almost entirely of endothelioid cells. Occasionally Langhans' giant cells are observed in the peripheral granulomatous zone. Bacterial colonies are rarely identified with the lesions in rabbits, but when found, as in Rabbit 4, they appear to occupy the central zone and are surrounded by an area of necrosis in which a few polymorphonuclear leukocytes are visible. The bacterial colonies are composed entirely of small, Gram-negative bacillary elements, without peripheral clubs.

It is easily possible to fulfil Koch's postulates by the rabbit inoculations. Pure cultures, as obtained from the lesions of the patient, caused the death of Rabbits 1 and 2 and produced in them lesions

TABLE III

Inoculation of Animals

Animal	Date	Material	Culture	Dosage	Method of inoculation	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
				cc.						
Rabbit 1	1/14/33	Liver, patient	24 hour pure brain broth	1.0	Intravenous	Severe general reaction	Died 1/15/33	1	Granulomatous foci liver, lungs, spleen	Liver and spleen
Rabbit 2	1/15/33	Liver, patient	48 hour pure brain broth	0.3	Intravenous	Slow general reaction	Died 1/30/33	15	Granulomatous foci liver, cecum, lungs, spleen, kidney	Spleen
Rabbit 3	1/20/33	Spleen, Rabbit 1	24 hour pure brain broth	0.8	Intravenous	Slow general reaction	Died 1/31/33	11	Granulomatous foci liver, lungs, spleen, kidney	Liver
Rabbit 4	1/25/33	Spleen, Rabbit 1	24 hour pure brain broth	1.5	Intravenous	Slow general reaction	Died 1/30/33	5	Granulomatous foci liver, lungs, spleen	Blood, liver, spleen
Rabbit 5	2/11/33	Spleen, patient	24 hour pure brain broth	2.5	Subcutaneous	Abscess site inoculation, no general reaction	Killed 3/14/33	31	Granulomatous foci lung, subcutaneous abscess	Subcutaneous abscess
Rabbit 6	2/11/33	Spleen, patient	24 hour pure brain broth	2.5	Subcutaneous	Abscess site inoculation, general reaction, rapid terminal failure	Died 2/18/33	7	Granulomatous foci liver, spleen, abscess site inoculation	Blood, liver, spleen, subcutaneous abscess
Mouse 1	1/12/33	Blood patient, ante mortem	Original 24 hour brain broth	0.2	Subcutaneous	Slight local induration, no general reaction	Killed 2/9/33	28	Granulomatous foci spleen	Accidentally lost
Calf 1	2/1/33	Spleen, patient	24 hour pure brain broth	5.0	Subcutaneous	Mild general reaction, fever 2 days, bloody diarrhea, local swelling, recovered	Killed 3/29/33	57	No lesions	Negative

similar to those revealed at autopsy of the patient. Pure cultures of the organism as inoculated were obtained from the liver and spleen. Rabbits 3 and 4 were inoculated with the culture that had been isolated from Rabbit 1, and there also the lesions were unmistakably almost identical with those observed at autopsy of the patient. The organism was again isolated from the lesions of these rabbits in pure culture and proved to be identical with the original culture. In only one of the intravenously inoculated rabbits, Rabbit 4, was the blood culture positive at the time of death.

Subcutaneous inoculation of two rabbits proved fatal in one, the rabbit dying on the 7th day of septicemia. A local abscess was produced, as well as lesions in the liver and spleen. The other rabbit that received subcutaneous inoculation survived and was killed on the 31st day after inoculation. It revealed a well defined encapsulated local abscess and a few healing granulomatous abscesses in the lungs. In the lesions of Rabbit 6 colonization of the organisms in the disseminate lesions was a prominent feature. In Rabbit 5 organisms were not identified in the tissue sections. In both, however, cultures were positive.

Mice: Judging from the inoculations of one white mouse (Table III) mice are practically non-susceptible. Subcutaneous inoculation did not appear to provoke any illness or local lesion. The animal was killed on the 28th day; a few small, typical, chronic granulomatous foci were found in the spleen only.

Cattle: One calf was inoculated and results were essentially negative (Table III).

Guinea Pigs: These animals exhibited a variable susceptibility, although in all but one some lesions were produced. The results of subcutaneous inoculations are summarized in Table IV. Although local lesions were produced in all but Guinea pig 2 it will be noted that none of the animals died from the infection. Guinea pig 8 died on the 16th day after inoculation; pneumonia and not *Actinobacillus* infection was the cause of death. The pigs usually manifested slight disability, chiefly refusing food and losing weight for a few days, then generally recovered. The most severe reactions were encountered in Guinea pigs 1 and 4. Both were very ill when they were killed and probably would have died as a result of actinobacillosis. The severity of the illness of Guinea pig 1 was accounted for at autopsy by general peritonitis.

TABLE IV

Subcutaneous Inoculation of Guinea Pigs

Guinea pig	Date	Material	Material inoculated	Dosage	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
1	1/12/33	Spleen, patient	Triturated abscess spleen	Saline suspension 1 cc.	Abscess site inoculation, severe general reaction	Killed 1/18/33	6	Granulomatous foci liver, spleen, lungs, subcutaneous abscess, general peritonitis	Subcutaneous abscess, peritoneum, liver, spleen, blood
2	1/12/33	Lung, patient	Triturated abscess lung	Saline suspension 1 cc.	Small abscess site inoculation, mild general reaction, recovery	Killed 3/14/33	61	No lesions	Negative
3	1/12/33	Blood, patient, ante mortem	Original 24 hr. brain broth ante mortem culture	Brain broth culture 1 cc.	Small abscess site inoculation, drainage 50th day, no general reaction	Killed 3/14/33	61	Granulomatous foci liver, lungs, subcutaneous abscess	Subcutaneous abscess
4	1/12/33	Liver, patient	Triturated abscess liver	Saline suspension 1 cc.	Large abscess site inoculation, severe general reaction	Killed 2/4/33	23	Granulomatous foci liver, spleen, lung, subcutaneous abscess	Negative (contamination)
5	1/25/33	Blood, patient, ante mortem	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess and ulcer site inoculation, mild general reaction	Killed 2/9/33	15	Granulomatous foci liver, spleen, lungs, cutaneous ulcer	Negative (cultures lost)
6	1/25/33	Liver, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess and ulcer site inoculation, mild general reaction	Killed 2/20/33	26	Granulomatous foci liver, lung, cutaneous ulcer	Negative
7	1/25/33	Spleen, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess site inoculation, mild general reaction	Killed 2/20/33	26	Granulomatous foci liver, cutaneous abscess	Subcutaneous abscess
8	1/25/33	Lung, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess site inoculation, severe general reaction	Died 2/10/33	16	Granulomatous foci liver, lymph node, spleen, cutaneous abscess, pneumonia	Subcutaneous abscess

Subcutaneous abscesses formed at the sites of inoculation in all of the animals except Guinea pig 2, in which lesions could not be found at autopsy. In two instances the abscesses began to drain after a few days and a large purulent ulcer with necrotic base and undermined borders formed. In Guinea pig 6 the ulcer began to heal about the 20th day and when the animal was killed, on the 26th day, the ulcer was almost healed. In Guinea pig 5, however, the ulcer remained about 2 cm. in diameter and appeared to be progressing at the time the animal was killed. In Guinea pig 3 a small abscess developed which persisted, and on the 50th day a small sinus appeared, through which purulent material drained. In the eight guinea pigs inoculated subcutaneously small, discrete, grayish white, necrotic foci were found in the livers of seven, in the lungs of five, and in the spleens of four. One of the guinea pigs had general peritonitis of serofibrinopurulent type and one had an enlarged inguinal lymph node containing necrotic foci.

Microscopically the lesions in these animals are similar to those in the rabbits. The local subcutaneous abscesses are composed centrally of a large area of necrotic exudate in which a few polymorphonuclear leukocytes are preserved, but usually only nuclear detritus and eosin-staining granular material are present. Surrounding this is a wide zone of well preserved polymorphonuclear leukocytes that blends peripherally with a zone of granulation tissue in which fibroblasts and young blood vessels form the supportive structure. Intermingled with these cells are polymorphonuclear leukocytes, large mononuclear phagocytic cells, plasma cells, lymphocytes and other cells, sometimes quite numerous, interpreted as endothelioid. In the outer portions bacterial aggregations exist in colony formation; these are Gram-negative and not associated with peripheral clubs. In the viscera the lesions tend toward chronic abscesses, with central collections of polymorphonuclear leukocytes and peripheral granulomatous reaction. In some of the lesions necrosis is a prominent feature, but in others it is slight. In the older lesions the peripheral zone has become distinctly fibrous, and in others this fibrosis has progressed to involve most of the focus, as though to form a cicatrix of a healed lesion. However, in the more severe general infections, as in Guinea pigs 1 and 4, all stages of lesions exist from focal areas of necrosis to abscesses, as described. Intermediate lesions frequently resemble endothelioid tubercles, or wide zones of

TABLE V

Intraperitoneal Inoculation of Guinea Pigs

Guinea pig	Date	Culture	Culture dosage 1 cc.	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
1	1/14/33	Spleen, patient	24 hour pure brain broth	Severe general reaction, conjunctivitis	Killed 1/18/33	4	Fibrinopurulent peritonitis, granulomatous foci in peritoneum, liver, spleen, lungs, lymph nodes	Liver, peritoneum, spleen
2	1/14/33	Spleen, patient	24 hour pure brain broth	Severe general reaction, conjunctivitis	Died 1/20/33	6	Fibrinopurulent peritonitis, granulomatous foci in liver, spleen, lungs, kidneys, lymph nodes	Spleen
3	1/25/33	Blood, patient	24 hour pure brain broth	Severe general reaction	Died 2/3/33	9	Fibrinopurulent peritonitis, granulomatous foci in liver, spleen, lungs	Liver, peritoneum
4	1/25/33	Liver, patient	24 hour pure brain broth	Severe general reaction	Died 1/31/33	6	Fibrinopurulent peritonitis, granulomatous foci in liver, spleen, lungs, adrenals, lymph nodes	Blood, liver, peritoneum
5	1/25/33	Spleen, patient	24 hour pure brain broth	Severe general reaction	Died 1/30/33	5	Fibrinopurulent peritonitis, granulomatous foci in liver, spleen	Blood, liver, peritoneum
6	1/25/33	Lung, patient	24 hour pure brain broth	Mild general reaction, recovery, subcutaneous abscess with draining sinus	Killed 2/20/33	26	Subcutaneous abscess with draining sinus, granulomatous foci in liver, lungs, adrenals, local peritonitis, periorchitis	Negative

central necrosis with a slight exudative and granulomatous proliferative reaction peripherally. In most instances colonies of bacteria are associated with the lesions; in the early lesions in the center of the necrotic focus, and in older lesions toward the border of the zone of necrosis or in the area of exudation. These are frequently found as large compact masses not retaining the Gram stain, and sometimes with just a faint suggestion of peripheral clubs.

Following intraperitoneal inoculation of guinea pigs with pure cultures (Table V), the animals became ill in from 24 to 48 hours; they refused food, were weak and listless, the hair became roughened, they lost weight and the abdomen became tender. All but two of the animals, Guinea pigs 1 and 6, died in from 5 to 9 days. Guinea pig 1 was moribund at the time it was killed and would have died within a few hours. Guinea pig 6, however, after an initial slight general reaction, apparently recovered with only a local abscess and draining sinus at the site of inoculation. It was killed on the 26th day. In none was the Strauss reaction elicited clinically. At autopsy all the animals were found to have peritonitis. In only one, Guinea pig 6, was the reaction local; it was confined to an area beneath the parietal peritoneum of the ventral wall, and adherent loops of small intestine and omentum. A sinus tract through the peritoneum and abdominal muscles formed a subcutaneous abscess and a draining sinus through the skin. The peritonitis was of a peculiar type. The peritoneal cavity contained a few cc. of clear amber fluid. The surface of the liver and spleen and usually the omentum and stomach were covered by a thick, adherent, grayish yellow, fibrinopurulent exudate. These structures were frequently adherent to one another. Over the coils of the small intestines there were frequently isolated or discrete, small, grayish yellow foci that resembled tubercles. In two guinea pigs (Guinea pigs 1 and 6) the same appearance was presented on the tunica of the testis and epididymis. The livers of all the animals were involved by fine grayish yellow points of apparent necrosis. Similar lesions, although sometimes more distinct, were observed in the lungs and spleen of five animals. In two animals the suprarenal glands were slightly involved. In one animal the mediastinal lymph nodes were involved and in one, Guinea pig 6, the inguinal nodes. In two animals, as has been noted, periorchitis existed.

Microscopically, except in Guinea pig 6, the lesions are usually relatively acute, but throughout the various involved organs and the

various animals, lesions of varying age are presented. These are observed in their simplest form as areas of necrosis at the point of lodgment of bacterial masses in the capillaries, to focal abscesses or focal granulomatous, tubercle-like structures. The early lesions consist of a central compact aggregation of bacteria surrounded by a zone of necrosis in which a few polymorphonuclear leukocytes are seen. Slightly older lesions reveal a peripheral zone of endothelioid cells, with perhaps a greater number of polymorphonuclear leukocytes collected around the bacterial colony (Fig. 5). In other instances lesions of this stage reveal only central necrosis with a rim of polymorphonuclear leukocytes and endothelioid cells, or with the central zone composed entirely of polymorphonuclear leukocytes without necrosis. Central or eccentric masses of bacteria are sometimes observed in the latter lesions, although they not infrequently are entirely absent. Still older lesions are in certain instances predominatingly endothelioid tubercles, sometimes with central polymorphonuclear neutrophilic exudation and at other times with almost solid endothelioid cells. In Guinea pig 6 the lesions are more chronic (26 days). They consist of central polymorphonuclear leukocytes with considerable necrotic cellular débris, a peripheral rim of endothelioid cells and fibroblasts or fibrous connective tissue. These lesions are in the liver, suprarenal glands, lungs, tunica of the testes and subcutaneous tissue. In these chronic lesions the masses of bacteria are not visible.

The Pathological Changes in Bovine Actinobacillosis

The lesions of bovine actinobacillosis are grossly like those of actinomycosis, but although true bovine actinomycosis usually involves the jaw bones, where it produces rarefying osteitis, bovine actinobacillosis usually affects the soft tissues of the head and neck. Specifically the parts most often affected are the tongue, palate, pharynx, cheeks, skin of face and lymph nodes of the submaxillary and cervical regions. The lungs have been described also as the site of affection.

Grossly the smaller bovine lesions may be described as appearing like areas of soft granulation tissue with central suppuration, the central zone of exudation gradually enlarging and the peripheral zone of granulation tissue gradually developing a thick fibrous capsule. The pus is thick, viscid or gelatinous and greenish yellow. In it the sulphur granules are found.

The microscopic characteristics of the lesions, as determined in bovine material from which Thompson isolated the *Actinobacillus* in pure culture, will be appended for comparative study. These corresponded with the previously published descriptions of such lesions. They also were essentially the same as those that were found in our patient, and those that were produced in our experimental animals. The central portion of the lesion, which was essentially a granulomatous abscess, was composed of exudate in which fairly well preserved polymorphonuclear leukocytes predominated. Small granules, the colonies of bacteria with peripheral clubs, were fairly abundant in the exudate that adhered to the wall of the abscess. This wall consisted of granulation tissue of loose texture, the principal supportive structure of which consisted of fibroblasts and newly formed blood vessels, throughout which were interspersed lymphocytes, large phagocytic mononuclear leukocytes, plasma and endothelioid cells. Polymorphonuclear leukocytes gradually diminished in number as the peripheral portions were reached, except for focal aggregations. The uniform structure of the wall of the abscess was somewhat altered by the presence of small abscesses, similar, although much smaller, to the central large one. This gave the entire wall more or less of an alveolar appearance. The smaller abscesses were composed of a central portion in which one to several compact bacterial aggregations with peripheral clubs existed. When these were stained by Gram's method only Gram-negative bacillary forms in compact aggregations with peripheral clubs were revealed. They were entirely devoid of Gram-positive branched filaments. Surrounding the bacterial colonies there were well preserved polymorphonuclear leukocytes, some necrosis and occasionally giant cells. Peripherally there was an encircling zone of slightly more compact granulation tissue than was found elsewhere in the wall of the large abscess, with a tendency to strands of fibrous connective tissue as a final encapsulation for the alveolar structure. Surrounding the entire lesion was a dense encapsulation of fibrous tissue.

Epidemiology

The patient's immediate family was carefully questioned concerning possible sources of infection, but without eliciting information of value. It was stated most emphatically that he had had no contacts with cattle or other animals, that he had never drunk milk as a

beverage and that all milk used at his home was pasteurized dairy milk. He had always insisted on his meats being well cooked. There had been no local injuries or lesions about the hands or other exposed surfaces of the body.

The method of natural spread of the disease among cattle is not known, but it is presumed that the organism is strictly parasitic and contact with infected animals or carriers constitutes the mode of dissemination. The disease usually occurs sporadically in cattle and is not highly contagious, although Lignières and Spitz report its occurrence in cattle in epizoötic form.

Summary

Three cases of actinobacillosis in man are now well authenticated. The case reported here offers the first opportunity for pathological study of the lesion in a human being. The lesions are essentially similar to those that have been described in cattle, except that in man the lesions are much more widespread, and sulphur granules, such as occur in bovine lesions, apparently do not occur. In the present case the lesions may be described as granulomatous abscesses severely affecting the lungs, liver and spleen. Lesions similar to those observed in man and cattle were produced in experimental animals. Glanders and tularemia were considered in the differential diagnosis, but both seemed to be definitely ruled out by the bacteriological and serological investigations. The organism isolated from our case reveals a close cultural and antigenic relation to *Actinobacillus lignièresi*, and a more distant relation to *Pfeifferella mallei* and *Bacillus whitmori*. Thompson has previously shown that *Actinobacillus lignièresi* of bovine and human origin, *Pfeifferella mallei* and *Bacillus whitmori* are antigenetically interrelated, and has proposed that they be included in a common genus. This relationship, as far as the organism in our case is concerned, is also confirmed from the pathological viewpoint, for the lesions in man and in experimental animals were similar to glanders, as well as exhibiting similarities to bovine actinobacillosis. Although the organism under consideration revealed minor cultural differences and somewhat different antigenetic phenomena from a typical strain of *Actinobacillus lignièresi* of bovine origin, nevertheless, it seems justifiable to regard the organism in this case as a variant of the usual bovine strain of *Actinobacillus lignièresi*.

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DESCRIPTION OF PLATE

PLATE 101

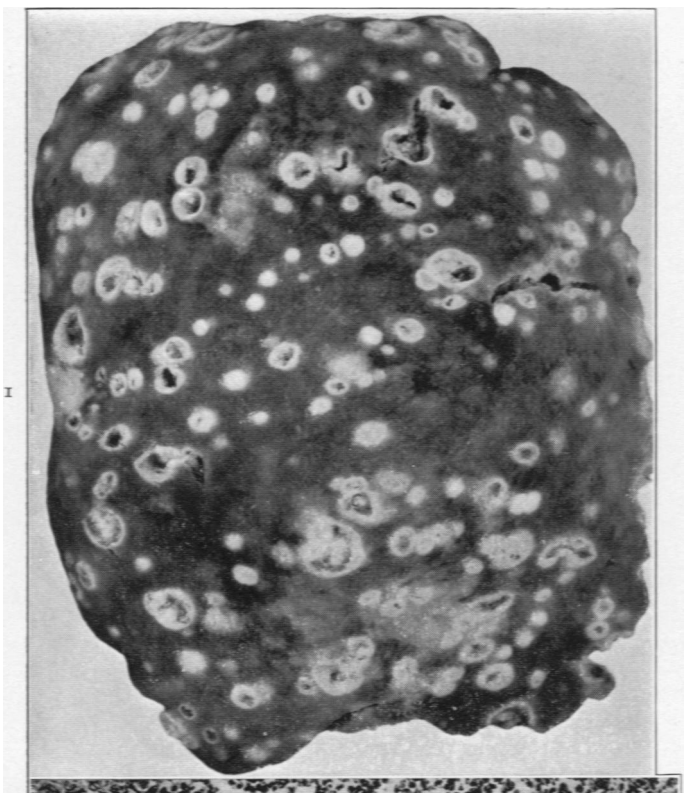
FIG. 1. Spleen of patient revealing multiple granulomatous abscesses.

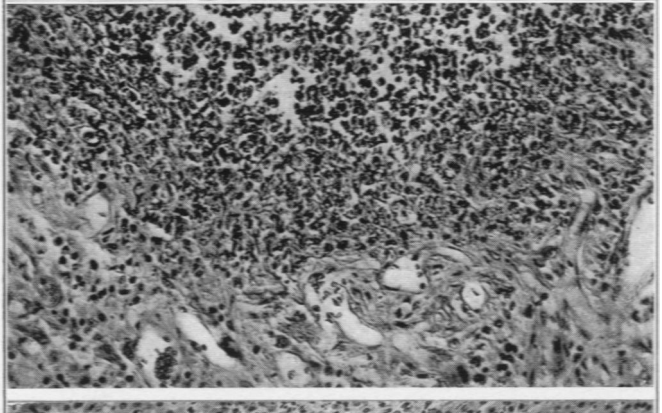
FIG. 2. Granulomatous abscess from lung of patient. In the center of the abscess polymorphonuclear neutrophilic leukocytes predominate with a few mononuclear phagocytes and lymphocytes. Peripherally endothelioid cells and fibroblasts are seen just within and intermingling with a granulation tissue encapsulation. Hematoxylin and eosin stain. × 160.

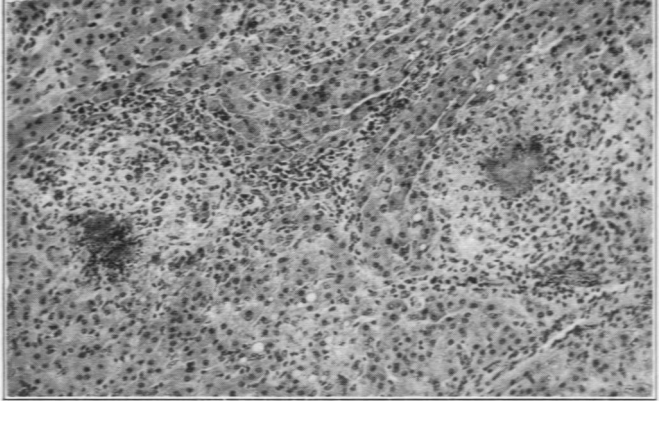
FIG. 3. Portion of large granulomatous abscess from liver of patient. Central necrosis, zone of leukocytes and early peripheral endothelioid cell proliferation may be seen. Large masses of bacteria in colony formation, more deeply stained, may also be seen. Hematoxylin and eosin stain. × 45.

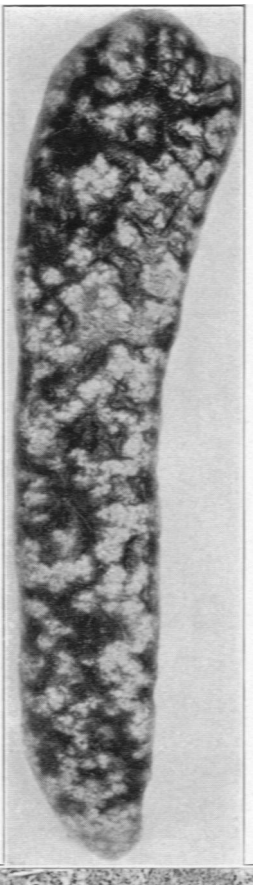
FIG. 4. Spleen of Rabbit 3 inoculated intravenously with pure culture of *Actinobacillus*, as isolated from the liver of the patient after passage through Rabbit 1. The rabbit died the 11th day after inoculation. The resemblance to the lesions in the patient's spleen may be noted (Fig. 1).

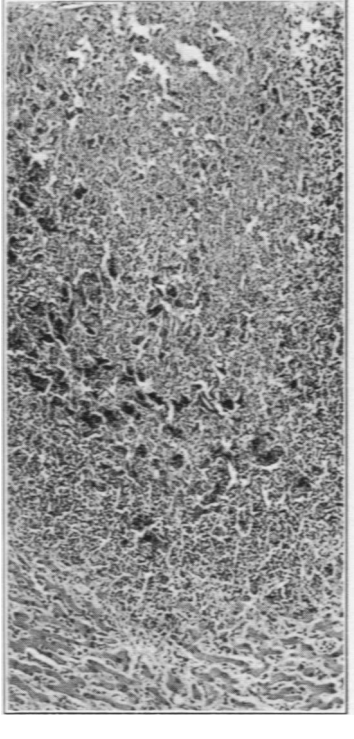
FIG. 5. Liver of guinea pig inoculated intraperitoneally. Two well defined lesions are revealed. Colonies of *Actinobacillus* may be noted in the centers of the lesions. Surrounding the bacterial colonies are polymorphonuclear neutrophilic leukocytes and necrosis, and at the periphery is a well defined zone of endothelioid cell proliferation. Hematoxylin and eosin stain. × 110.











Beaver and Thompson

Actinobacillosis of Man