A STUDY OF BACILLUS PYOGENES.

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Plates 14 to 16.

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INTRODUCTION.

Bacillus pyogenes is associated with various disease processes of swine and cattle and is not infrequently found in milk. Failure to recognize it as the *B. pyogenes* described in French, Dutch, and German literature may be due to certain difficulties in its cultivation, to its close resemblance to streptococci under certain conditions, and to the fact that it is usually found in mixed culture with organisms which may mask its presence.

In France, according to Lucet (1893), next to the streptococci *B. liquefaciens pyogenes* is one of the most frequently found organisms in suppurations of cattle. In Germany Künnemann (1903) found a similar organism, which he calls *B. pyogenes bovis*, in 90 per cent of suppurations of cattle. Grips (1898) found *B. pyogenes suis* commonly present in pleuritis and peritonitis of swine. Tuff (1906) found *B. pyogenes* in over 13 per cent of milk samples examined. Glage (1902–03) found the organism third in importance to streptococci and *B. tuberculosis* as a cause of mastitis in cows. Eggink is quoted by Ward (1917, *a*) as having found *B. pyogenes* of first importance in metritis of cattle. There are no statistics as to the prevalence or distribution of the organism in America. Ward (1917, *a*) found it frequently in swine and cattle.

Lucet (1893) studied 52 cases of suppuration, cold abscesses, traumatic abscesses, and cases of septicemia, all in cows. He says: It seems that there exist in the cow special pyogenic microbes, not yet described, which are a streptococcus, a staphylococcus, and three bacilli. He names these organisms *Streptococcus pyogenes bovis*, *Staphylococcus pyogenes bovis*, *B. pyogenes bovis*, *B. liquefaciens pyogenes bovis*, and *B. crassus pyogenes bovis*. His description of *B. liquefaciens pyogenes bovis* agrees with that of the *B. pyogenes* of Grips, Glage, Künnemann, and others. Lucet gives a photograph of the organism and describes it as non-motile, liquefying gelatin slowly, not growing on potato, growing as a sediment in veal bouillon without producing turbidity, non-virulent for guinea pigs, injected into rabbits intravenously producing subaponeurotic abscesses principally in the limbs where they sometimes acquire great size but do not discharge. His *B. pyogenes bovis*

resembled the above morphologically but did not liquefy gelatin and its pathogenicity for guinea pigs was variable. *B. crassus pyogenes bovis* was a larger motile bacillus. Grips (1898) described *B. pyogenes suis* and the lesions of the pleura and peritoneum from which it was isolated. Poels (1899) described a similar organism from polyarthritis of calves and called it the polyarthritis bacillus. Künnemann (1903) described *B. pyogenes bovis* from suppurations of cattle. Glage (1902-03) made a careful comparison of *B. pyogenes suis* (Grips) and *B. pyogenes bovis* (Künnemann) and concluded that they were identical. He proposed that the organism be called *B. pyogenes*. His contention that the organisms from swine and cattle are identical has not been seriously questioned. Careful bacteriological studies have been made by Koske (1906), Berger (1908), and Holth (1908). Ward (1917, *a* and *b*) has given valuable brief summaries in English, and Glage (1913) in German.

Source of Strains.

The strains of *Bacillus pyogenes* of bovine origin employed in this study were received from Dr. Theobald Smith who supplies the following data concerning their source.

Strain I. From pneumonic lungs of a calf. Killed when 33 days old.[1]

Strain II. From pneumonic lungs of a calf. Killed when about 5 weeks old.[1]

Strain III. From pneumonic lungs of a calf. Killed when 38 days old.[1]

Strain IV. From pus filling both horns and body of uterus of a cow. Uterus obtained after slaughter of cow.

Strain V. From a similar case as that of Strain IV. Uterus contained a foul smelling fluid.

Strain VI. From purulent contents of uterus in case of prolapse of vagina and external os. Uterus obtained when cow was slaughtered.

Strain VII. From pneumonic lungs of a calf. Killed when 31 days old.

Strain VIII. From the uterine contents and ovaries of a case of purulent metritis and of central necrosis and pus formation in both ovaries. Associated with *B. actinoides*.

Strain IX. From chocolate-colored, offensive fluid contained in uterus of a cow slaughtered. Other bacteria present.

Strain X. From kidney, liver, and lungs of the fetus of Cow 259. Several other species of bacteria present in small numbers.[2]

Strain XI. From the fourth stomach, liver, and lungs of the fetus of Cow 291.[2]

Strain XII. From the liver of the fetus of Cow 339. Pure culture of *Vibrio fetus* isolated from the lungs.

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[1] It is highly probable that in these cases *Bacillus pyogenes* was secondary to *Bacillus actinoides* (Smith, T., *J. Exp. Med.*, 1918, xxviii, 333).

[2] Smith, T., *J. Exp. Med.*, 1919, xxx, 325.

Strain S 1 was isolated by Dr. Carl TenBroeck from the pneumonic lung of a case of hog-cholera. Other organisms were also present in the lung.

Cultural Study.

The known morphological and cultural characteristics of *B. pyogenes* as described by Lucet (1893), Grips (1898), Poels (1899), Künnemann (1903), Glage (1902–03), Koske (1906), Berger (1908), and Holth (1908) have been summarized by Glage (1913), Buchanan and Murray (1916), and Ward (1917, *b*). They are briefly as follows:

The organism is a small slender rod 0.2 to 3.0 microns in length by 0.2 to 0.3 microns in thickness. It is quite pleomorphic being often coccoid, club-shaped, or slightly curved. It is non-motile and produces no spores. Some authors (Glage, Künnemann) have regarded it as Gram-negative and others as Gram-positive (Berger, Holth, Olt, Ward). Berger found it Gram-positive if subjected to sufficient exposure to the iodine solution. Capsules are not produced.

It is stated by most authors that the organism grows very poorly or not at all in standard bouillon or on standard agar, and that it requires hemoglobin, blood, or serum in the medium. Good growth occurs on blood or serum agar. Coagulated blood serum is slowly liquefied beginning in about 48 hours as small depressions underlying each colony. Because of this characteristic this medium has been a favorite one for isolating the organism. Milk is coagulated in about 48 hours and the curd is subsequently slowly dissolved or digested. In liquid serum or serum bouillon growth occurs in the form of a sediment. Growth does not occur at temperatures below 24°C. but in a specially prepared nutrient gelatin of high melting point Poels found liquefaction produced by the organism growing at 26°C. Growth occurs under aerobic and anaerobic conditions. Gas is not produced in carbohydrate media. According to Pütz (1904) acid is produced. Koske (1906) reports acid production in serum litmus whey. Berger (1908) obtained no growth in serum litmus whey and does not mention acid production in lactose or dextrose bouillon but notes that milk is coagulated and soured. Holth (1908) reports acid production from dextrose, fructose, galactose, maltose, lactose, and saccharose in a special meat extract (Cibil's) bouillon but obtained no acid or visible growth in the same medium containing xylose, rhamnose, arabinose, sorbose, mannitol, sorbitol, dulcitol, or glycerol. Indole, hydrogen sulfide, and nitrites are not produced. Methylene blue, litmus, and neutral red are not reduced. The bacillus is soon killed at 57°C. and is very sensitive to antiseptics.

Our experience with the organism agrees with the above as regards morphology, oxygen requirements, growth on coagulated serum, in milk, and in serum bouillon. In plain standard veal infusion bouillon made with Fairchild's peptone, however, we have obtained fairly

good growth, at least with strains which have been in cultivation a very short time. For a while the bouillon was not clouded by the culture but after cultivation for several months bouillon was distinctly clouded in 24 hours by most strains.

The production of hemolysis in blood agar by *Bacillus pyogenes* appears not to have been noted heretofore. In standard veal infusion agar plus 5 to 10 per cent of defibrinated horse blood there appear after incubation for 20 to 24 hours very small zones of hemolysis about very minute deep colonies. The colonies are often visible only under the low power of the microscope. In 48 hours the deep colonies are still quite small biconvex discs about 0.3 mm. in greater diameter but are easily seen macroscopically. The hemolyzed zones are clear, well defined, colorless, and of the beta type (Smith and Brown, 1914–15; Brown, 1919), about 1.5 to 2 mm. in diameter (Fig. 1). Isolated surface colonies do not appear so readily on the plate under aerobic conditions and the zone of hemolysis may be hardly visible. If the plate is sealed, however, individual surface colonies grow more readily and produce zones of hemolysis similar to those of deep colonies. If the blood agar plate is streaked so that many small surface colonies appear in the line of the streak, hemolysis appears beneath the streak. The individual surface colonies are very small convex colorless droplets much like those of *Bacillus influenzæ*.

We have sought to determine whether *Bacillus pyogenes* is hemoglobinophilic or whether it may be dependent upon other substances in blood for growth.

Freshly drawn horse blood was allowed to clot and a clear straw-colored serum was obtained as nearly free from hemoglobin as possible. This was used in serum agar plates.

Another portion of the same blood was defibrinated, and the corpuscles were washed repeatedly with sterile physiological salt solution. Some of the washed corpuscles were used in washed corpuscle agar plates.

Some of the washed corpuscles were laked with sterile distilled water and the corpuscle stroma removed by centrifugation. Care was taken to centrifuge the laked blood corpuscles until the supernatant hemoglobin solution no longer gave a clouding reaction with salt (Brown, 1919[3]). The hemoglobin solution so obtained was used in hemoglobin agar plates.

[3] Brown (1919), p. 67.

The corpuscle stroma obtained by centrifugation of the laked corpuscles was washed repeatedly in sterile distilled water and in salt solution until no visible trace of hemoglobin remained. The stroma suspension was used in stroma agar plates.

Each strain of *Bacillus pyogenes* was inoculated into the depths and streaked onto the surface of plates of the following media: (1) blood agar; (2) serum agar; (3) washed corpuscle agar; (4) hemoglobin agar; (5) stroma agar; (6) plain agar. Every precaution was taken to insure uniformity of conditions, such as use of the same lot of agar throughout, inoculation of one plate after another in the same manner and with the same amount of material. The plates were inoculated from fresh plain bouillon cultures. Observations were made as to the amount of growth, size and number of colonies, and morphology of the organisms within the colonies. In every case the best growth was obtained in blood agar. Next best was the growth in washed corpuscle agar and in serum agar. In the majority of cases better growth was obtained in stroma agar than in hemoglobin agar. Little or no growth occurred in plain agar. The results indicate that serum is of as much importance as corpuscles, and that hemoglobin is probably the least essential of the blood constituents for the growth of this organism. That hemoglobin does not satisfy the requirements of *Bacillus pyogenes* as it does those of *Bacillus influenzæ* is indicated by the following experiment. A blood agar plate was inoculated in the depths and also streaked with *Bacillus pyogenes*. At a point near the streak a large zone of laking was produced by depositing a bit of saponin on the surface of the medium. Both deep and surface colonies within the zone were no larger and no more numerous than elsewhere in the plate (Fig. 1). Under similar conditions colonies of *Bacillus influenzæ* grew more luxuriantly in and near the zone of laking than elsewhere in the plate. A similar result was obtained by producing zones of laking by directing a stream of carbon dioxide against the bottom of the plate until a spot was frozen. The blood used for such experiments must be fresh; otherwise there will be sufficient free hemoglobin in the serum to obliterate the difference in growth between that in the laked zone and elsewhere.

The fact that colonies of *Bacillus influenzæ* grow more luxuriantly in the vicinity of colonies of hemolytic streptococci and staphylococci has

been known for many years (Grassberger, 1897). According to Davis (1917) there is involved in this phenomenon not only hemoglobin but also a vitamine which is supplied by the foreign organism or may be supplied by fresh sterile vegetable or animal tissues. We have not found the growth of *Bacillus pyogenes* colonies to be augmented by proximity to colonies of other organisms.

That blood is not absolutely necessary for the growth of *Bacillus pyogenes* is shown by the fact that it grows very well on Dorset's egg medium. On this medium the colonies, especially those on the upper half of the slant, produce little pits like those produced on coagulated serum. The organism also grows fairly well on a medium consisting of three parts of white of egg plus one part of standard veal infusion bouillon, slanted and coagulated in the inspissator. On this medium the streak of growth also produces some depression as on coagulated serum. Even an old laboratory strain of *Bacillus influenzæ* which has become quite easy to cultivate grows very slightly on Dorset's medium and not at all on the egg white medium.

Staining and Morphology.

Bacillus pyogenes stains well with dilute carbolfuchsin or with Löffler's methylene blue but we have obtained the best results with the Gram stain. Under the influence of prolonged decolorization with alcohol the organism may not retain the violet stain so tenaciously as do staphylococci, but when stained on the same slide with *Bacillus influenzæ*, *Bacillus coli*, the meningococcus, or other recognized Gram-negative organisms there can be no doubt as to *Bacillus pyogenes* being clearly Gram-positive. The disagreement in the literature as to the Gram staining of the organism may be due to the many methods in use for applying and making up this stain and the poor keeping qualities of some of them. It is also pointed out by Olt (1908) that the dead organisms found in old exudates are Gram-negative whereas the living organisms are Gram-positive. We have used Stirling's aniline gentian violet, but 1 or 2 per cent of the dye by weight rather than 5 per cent has been found sufficient. Exposure of films to the violet stain for 10 seconds, Lugol's solution for 15 to 30 seconds, decolorization in alcohol until no more color appears to be given off,

and counterstaining for 10 seconds in aqueous safranine has always given good results.

The various media employed have afforded opportunity to observe the pleomorphism of *Bacillus pyogenes*. Four or five more or less distinct forms and their intermediate stages are recognizable.

1. Bacillary Form.—These are small, short, homogeneously stained Gram-positive bacilli occurring singly (Fig. 2).

2. Fusiform Form.—In this form there are one or two strongly Gram-positive central granules while the ends of the bacilli fade out and take the counterstain to some extent. These are often slightly curved, and superficially resemble *Bacillus acne* though smaller (Figs. 3 and 4).

3. Diphtheroid Form.—These are bacilli of irregular length and contour, some clubbed, containing deeply stained bands or granules irregularly placed (Fig. 6).

4. Streptococcoid Form.—These appear exactly as Gram-positive diplococci and streptococci in small clumps and short crooked chains. If, as may be supposed, these cocci are but the granules of the diphtheroid form which have assumed a very regular size, form, and arrangement, the matrix forming the remainder of the bacillus is invisible to the eye though the photographs reveal a faint matrix which probably retained a trace of the red counterstain (Figs. 7 and 8).

5. Filamentous and Branching Form.—The bacilli appear drawn out with irregular diameter. There are few definite deeply staining granules but in some filaments there may be a deep violet portion blending gradually with a faintly stained or Gram-negative portion. Definite buds and branches were occasionally found, especially in Strain IV (Figs. 5 and 6).

Cultures often show a mixture of the above forms. Some strains have more of a tendency to assume one form and others another, but all the strains studied have produced all the various forms at one time or another. The streptococcoid form may be so definite and so habitual with certain strains that the organism may be mistaken for a streptococcus. We have spent some weeks studying what was thought to be a very unusual minute hemolytic streptococcus only to discover that we were working with *Bacillus pyogenes*.

In observing the morphology of the organism on various media we hoped to discover the factors which determined the variations in form, but the hope was only partially realized. In the lesions of animals the organism appears in the bacillary form with a tendency to be more granular in older lesions. In bouillon and serum bouillon the bacillary form predominates though any or all of the other forms may also be present; the streptococcoid form is least likely to appear. The latter form was encountered most commonly on washed corpuscle agar, stroma agar, and especially on hemoglobin agar. On blood agar there was a mixture of bacillary, granular, and streptococcoid forms. The growth on serum agar was bacillary. Long filamentous and branching forms were obtained in serum bouillon and in the condensation fluid of coagulated blood serum. Fusiform bacilli appeared most commonly in milk and in bouillon containing fermentable sugar. Our study has produced the impression that the filamentous and streptococcoid forms represent the two extremes of pleomorphism. The strains which produced filamentous forms most readily, *e.g.* Strain IV, produced streptococcoid forms with difficulty, while other strains, *e.g.* Strain X, produced streptococcoid forms readily but filamentous forms rarely. The forms most commonly encountered are the bacillary and fusiform.

Fermentation.

In fermented bouillon plus 10 per cent of sterile horse serum and 1 per cent of the test substance all the strains of *Bacillus pyogenes* produced acid from dextrose, saccharose, lactose, and xylose but not from raffinose, inulin, mannitol, and salicin. Holth (1908) reports that galactose, fructose, and maltose are also fermented. We differ from him in regard to xylose. Our fermented bouillon containing the test substances was probably more favorable for the growth of the organism than was his meat extract bouillon.

Fermentation tubes were employed for the tests and the contents of both the open bulb and the closed arm were titrated for acid after incubation of the cultures for 7 days. The results recorded in Table I show that the acidity of the bulb was much higher than that of the closed arm, little or no acid often being produced in the latter. *Bacillus pyogenes* is completely agglutinated by normal horse serum in

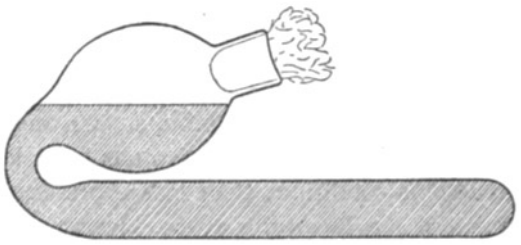
dilution of 1:100. In dextrose serum bouillon the arm of the fermentation tube remains clear and most of the growth is in the form of a sediment in the neck of the tube. If, however, the fermentation tube be incubated in the horizontal position (Text-fig. 1) an abundance of

TABLE I.

Fermentation Reactions.

Strain No.	Xylose.		Dextrose.		Lactose.		Saccharose.		Raffinose.		Inulin.		Mannitol.		Salicin.	
	Bulb.	Arm.	Bulb.	Arm.	Bulb.	Arm.	Bulb.	Arm.	Bulb.	Arm.	Bulb.	Arm.	Bulb.	Arm.	Bulb.	Arm.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	3.85	2.15	3.35	1.4	4.25	2.45	1.95	0.8	0.9	0.4	1.1	0.75	1.5	0.55	1.1	0.9
II	4.1	1.9	3.55	2.1	5.35	2.1	3.25	1.9	0.75	0.35	0.9	0.5	0.65	0.75	0.7	0.7
III	3.8	2.05	3.35	1.8	5.5	3.05	2.3	1.45	1.0	0.65	0.6	0.6	1.0	0.65	0.7	0.6
IV	4.0	1.9	3.9	1.2	3.35	0.85	2.75	0.9	0.85	0.4	0.75	0.55	1.0	0.65	0.75	0.4
V	3.55	2.2	3.7	1.95	3.9	1.65	2.8	1.6	1.15	0.6	0.9	0.4	0.75	0.5	0.7	0.4
VI	4.8	2.55	3.4	2.1	2.0	1.6	2.65	1.05	0.95	0.55	1.05	0.7	0.8	0.4	0.65	0.65
VII	3.35	2.55	2.6	2.2	4.05	2.35	3.75	1.65	1.2	1.0	1.3	1.05	1.15	0.08	1.6	0.85
VIII	4.4	3.2	4.0	1.5	4.6	2.5	3.2	2.7	0.65	0.45	1.0	0.9	0.95	0.5	1.1	0.65
IX	2.9	1.2	2.65	1.45	1.85	1.1	2.85	1.85	0.5	0.4	0.8	0.4	0.4	0.5	0.75	0.45
X	3.1	0.7	3.4	0.85	3.9	1.4	3.25	1.35	0.85	0.5	0.85	0.35	0.6	1.2	0.65	0.5
XI	3.75	1.8	3.85	1.5	2.8	1.05	3.4	1.15	1.1	0.8	1.2	0.4	0.75	0.45	0.8	0.6
XII	3.35	2.35	3.0	1.35	1.8	1.2	3.5	1.3	1.0	0.65	0.9	0.55	0.75	0.7	0.9	0.65
S 1	3.75	1.35	4.15	1.4	4.1	1.25	1.8	0.6	1.45	1.0	1.2	0.6	0.6	0.4	1.0	0.9

The figures indicate per cent normal total titratable acid.



TEXT-FIG. 1. Fermentation tube placed in the horizontal position.

acid is formed in the arm as well as in the bulb. These facts suggested for the moment that failure to ferment in the arm might be due to the mechanical effect of agglutination. It was discovered, however, that in plain dextrose bouillon without serum the arm was well clouded by

growth but little or no acid was produced in it. The effect of difference in oxygen tension was next considered. The closed arm of a fermentation tube of sterile bouillon containing 1 per cent of a 1:1,000 aqueous solution of methylene blue reduced by autoclaving remains colorless for weeks if incubated in a vertical position. The arm of a similar tube incubated in the horizontal position is well colored in a few hours. In the following experiment tubes of dextrose bouillon covered by 5 cc. of vaseline were placed in a boiling water bath for 30 minutes at the end of which time the methylene blue in the control tube was colorless. The other sealed tube was cooled and inoculated by means of a capillary pipette through the layer of vaseline. There was good growth in both inoculated tubes.

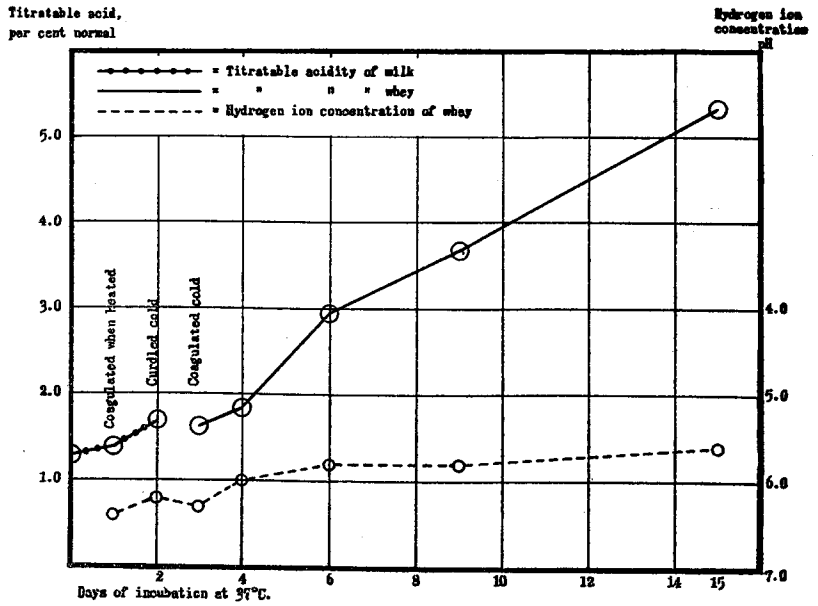
Strain No.	Sugar.	Relation to air.	Incubated 7 days.	
			Titration.	pH
			per cent	
X	Dextrose.	Not sealed.	3.9	5.1
	"	Sealed.	2.1	6.2
Sterile methylene blue.	"	"	Remained colorless.	

The titratable acidity is expressed as per cent normal acid.

It is to be noted that the behavior of *Bacillus pyogenes* as regards fermentation of sugars in relation to oxygen tension appears at variance with that of other organisms, notably those of the colon group. The idea, possibly correct in the case of most organisms, is prevalent that facultative anaerobic organisms are able to satisfy their oxygen requirements by breaking down fermentable sugars and that in such cases fermentation is likely to be more vigorous or at least more apparent under anaerobic conditions than in the presence of free oxygen. Some strains of *Bacillus coli* may produce an alkaline reaction in the bulb and acid and gas in the closed arm of the fermentation tube containing saccharose bouillon. *Bacillus cloacæ* may react similarly in lactose bouillon. The alkalinity or lower acidity of the bulb, however, may not be due to diminished fermentative activity but to the simultaneous production of large amounts of alkali.

Growth in Milk.

One of the cardinal cultural characteristics of *Bacillus pyogenes* is its ability to coagulate milk and slowly digest the curd. If an indicator solution such as rosalic acid and china blue (Bronfenbrenner, 1918–19) or bromocresol purple (Clark and Lubs, 1917, *b*) is placed in the milk, the latter is seen to become acid and remain acid for at least 3 weeks during which time much of the coagulum disappears. The question arises as to whether the coagulation of milk is due to acid or to an enzyme.



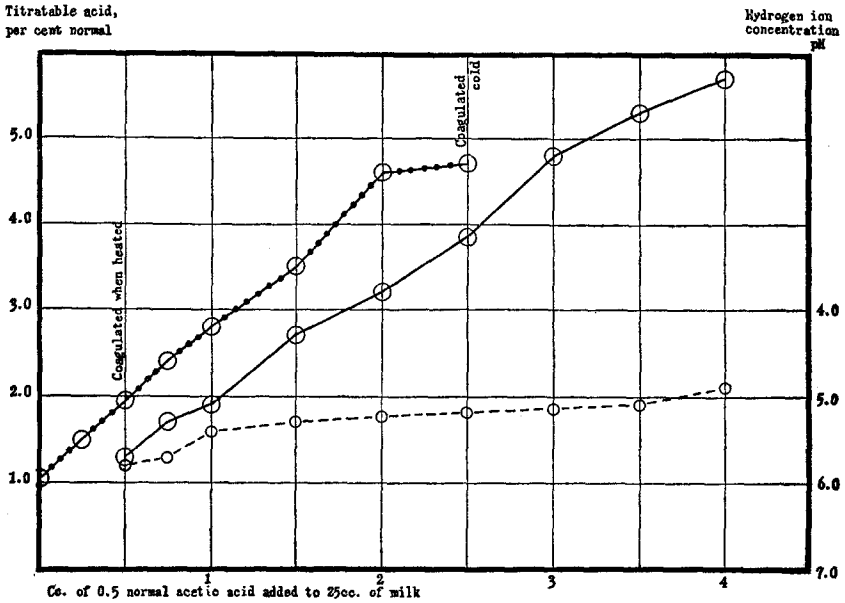
TEXT-FIG. 2. Titratable acidity and hydrogen ion concentration of fat-free milk after inoculation with *B. pyogenes*.

Seven tubes of fat-free milk were inoculated with *Bacillus pyogenes*. A tube was withdrawn for titration of total acidity and determination of hydrogen ion concentration[4] after incubation for 1, 2, 3, 4, 6, 9, and 15 days. The results are plotted in Text-fig. 2. The titratable acidity increased steadily. The hydrogen ion concentration of the whey

[4] Determinations of hydrogen ion concentration were made by the colorimetric method of Clark and Lubs (1917, *a*).

increased very little after the 6th day and reached a maximum of pH 5.6 in 15 days. The milk showed visible coagulation without application of heat on the 2nd day of incubation when the hydrogen ion concentration of the whey was pH 6.2 and the titratable acidity of the curdled milk was 1.7 per cent normal.

For comparison with these results tubes of milk were acidified with increasing amounts of 0.5 N acetic and hydrochloric acids. The results of titration of total acidity and determination of hydrogen ion concentration are plotted in Text-figs. 3 and 4.

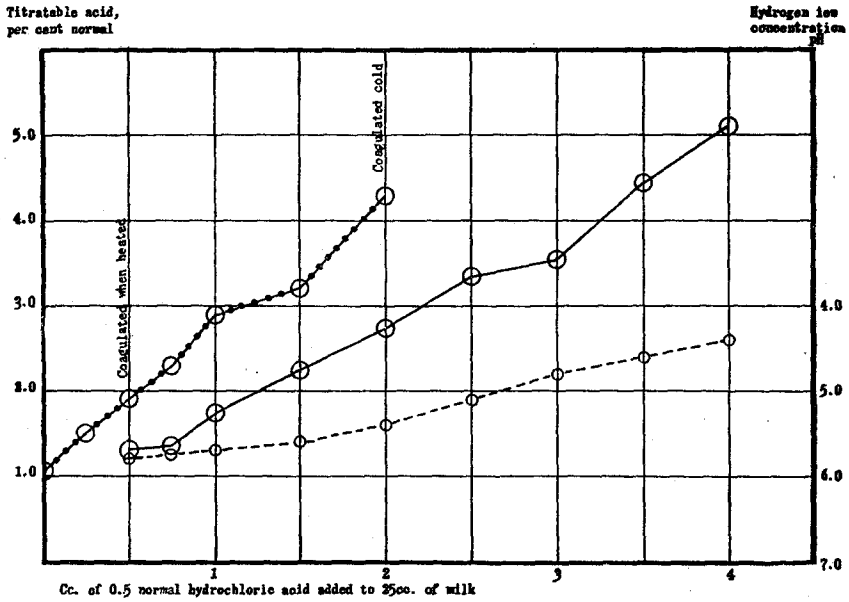


TEXT-FIG. 3. Total acidity and hydrogen ion concentration of fat-free milk after the tubes had been acidified with increasing amounts of acetic acid.

As was to be expected the hydrogen ion concentration showed more rapid increase with addition of hydrochloric acid than with similar amounts of acetic acid. With both the strong and the weak acid, however, the milk was coagulated without application of heat when it had reached a titratable acidity of 4.3 and 4.7 per cent normal respectively and the whey had a hydrogen ion concentration of pH 5.4 to 5.2. As was also to be expected the titratable acidity of the weak acid accompanying a hydrogen ion concentration sufficient to coagu-

late the milk was higher than that of the strong acid, the latter being more highly ionized. The difference is even more apparent in the titratable acidity of the whey.

In fermented bouillon plus 0.2 per cent of calcium chloride and 2.0 per cent of sodium caseinate *Bacillus pyogenes* produced a casein coagulum or sediment within 24 hours at 37°C. In the absence of calcium chloride no coagulation occurred. The same media gave similar results with rennet. These tests were made in media of four different reactions; namely, pH 6.2, 6.8, 7.1, and 7.7. After inoculation



TEXT-FIG. 4. Total acidity and hydrogen ion concentration of fat-free milk after the tubes had been acidified with increasing amounts of hydrochloric acid.

with *Bacillus pyogenes* and incubation for 20 hours, during which time coagulation occurred in all of those containing calcium chloride, the reactions were pH 6.2, 6.5, 6.7, and 7.2 respectively. The sedimented coagulum in the last tube was the most voluminous and the supernatant fluid from this tube gave no further precipitate when acidified with acetic acid. In the tube with pH 6.2 the sediment was more compact than in the others but acidification of the supernatant fluid showed that some casein was still in solution. It has also been found

that when *Bacillus pyogenes* is grown in fermented bouillon, plain bouillon, serum bouillon, dextrose bouillon, or dextrose serum bouillon plus sodium caseinate, the medium becomes very cloudy, almost opaque, in 24 hours and then clear again in another day or two. No sediment is formed or at most only a sediment of the organism itself. When the medium clears, however, the casein has disappeared and cannot be precipitated by acetic or nitric acid. Serum does not completely disappear and can be precipitated by nitric acid in cultures more than a week old. Apparently in the dextrose bouillon the casein is digested before the acidity rises sufficiently to precipitate it.

The preparation of casein calcium bouillon is attended with some difficulty. The method employed was as follows: To the sterile fermented bouillon add sufficient sterile 10 per cent calcium chloride to make a 0.2 per cent solution. Add sufficient sterile hydrochloric acid to dissolve the calcium phosphate which has been precipitated. At this point the medium reacts pH 6.2 to 6.4. Add sufficient 10 or 20 per cent sterile casein solution to make 2.0 per cent of casein in the bouillon. After the casein has been added the medium may be made alkaline if desired by the addition of sterile sodium hydrate. The calcium is apparently held by the casein so that it is not again precipitated as phosphate in the presence of a moderate amount of alkali. The preparation of this medium could probably be simplified by the use of calcium caseinate rather than sodium caseinate.

Since milk is coagulated by *Bacillus pyogenes* at a titratable acidity far below that required of hydrochloric or acetic acid and also at a much lower hydrogen ion concentration, and since soluble casein is coagulated in a neutral or slightly alkaline sugar-free medium, it appears that the coagulation of milk by *Bacillus pyogenes* is an enzyme rather than an acid coagulation. It is also to be noted that the casein is digested even in the presence of an excess of fermentable sugar. The process of digestion, however, seems to stop short of ammonia production since in a milk culture incubated for 3 weeks no increase in ammonia could be detected by Folin's method.

Immunological Study.

Four representative strains of *Bacillus pyogenes* were selected for the immunization of rabbits. Strain IV was selected because it showed slight morphological differences from the others. Strains VII, VIII, and X were selected because they were isolated from different lesions; *i.e.*, lungs, uterus, and a fetus, respectively. Rabbits

TABLE II.

Cross-Precipitation of Strains IV, VII, VIII, and X.

Strain No.	Serum No.	Dilutions.					Controls.	
		1:10	1:20	1:40	1:80	1:160	Normal rabbit serum, 1:10.	Salt solution.
IV	IV	++++	+++	++	++	+	−	−
	VII	++++	++	+	−	−	−	−
	VIII	++	Sl.	−	−	−	−	−
	X	++++	++	+	−	−	−	−
VII	IV	++	++	+	Sl.	−	−	−
	VII	++++	++++	++	−	−	−	−
	VIII	+	−	−	−	−	−	−
	X	++++	++	+	Sl.	−	−	−
VIII	IV	++	+	Sl.	−	−	−	−
	VII	++++	+++	+	Sl.	−	−	−
	VIII	++	+	+	+	Sl.	−	−
	X	+++	++	++	+	+	−	−
X	IV	++	+	Sl.	−	−	−	−
	VII	+++	++	+	+	−	−	−
	VIII	+	+	Sl.	−	−	−	−
	X	++++	+++	++	+	−	−	−

In the tables + + + + indicates maximum precipitation; Sl., slight precipitation.

were given at first several series of subcutaneous and intravenous injections of killed cultures, and later increasing amounts of living bouillon cultures intravenously at intervals of about 1 week. The rabbits tolerated the injections well and though all eventually succumbed to *Bacillus pyogenes* infection noticeable lesions did not develop until after the immune serum had been secured.

TABLE III.

Precipitation of All Strains by Sera IV and X.

Strain No.	Serum No.	Dilutions.					Controls.	
		1:10	1:20	1:40	1:80	1:160	Normal rabbit serum, 1:10.	Salt solution.
I	IV	++	+	Sl.	−	+	−	−
	X	+++	++	++	+	Sl.	−	−
II	IV	+++	+++	+	Sl.	−	−	−
	X	++++	+++	++	+	+	−	−
III	IV	++	+	+	Sl.	−	−	−
	X	++++	++	+	−	−	−	−
IV	IV	++++	+++	++	++	+	−	−
	X	++++	++	+	−	−	−	−
V	IV	++	++	+	−	−	−	−
	X	++++	+++	++	±	−	−	−
VI	IV	+++	++	+	−	−	−	−
	X	++++	++++	+++	++	+	−	−
VII	IV	++	++	+	Sl.	−	−	−
	X	++++	++	+	"	−	−	−
VIII	IV	++	+	Sl.	−	−	−	−
	X	+++	++	++	+	+	−	−
IX	IV	++	+	Sl.	−	−	−	−
	X	+++	++	+	−	−	−	−
X	IV	++	+	Sl.	−	−	−	−
	X	++++	+++	++	+	−	−	−
XI	IV	+	Sl.	−	−	−	−	−
	X	++++	++	+	−	−	−	−
XII	IV	++	++	+	Sl.	−	−	−
	X	++++	++++	+++	++	+	−	−

The titration of agglutinins was unsatisfactory because all but one or two strains were agglutinated to a considerable extent by normal rabbit serum in dilutions of 1:100 or 1:1,000. There was relatively little agglutination in salt solution controls. By regarding as positive only those tubes in which agglutination was stronger than in the normal serum controls it was evident that the immune sera produced agglutination of the homologous strains and many others in dilutions of 1:800 to 1:3,200. However, the normal agglutinins were such a disturbing factor that it is considered unsafe to draw any conclusions as to the relationship of the various strains on the basis of agglutination.

More satisfactory results have been obtained by titrating the precipitins. The precipitinogen used in the titrations consisted of the clear supernatant fluid obtained by centrifuging bouillon and blood bouillon cultures after incubation for 1 month. During incubation the cultures were frequently shaken. The blood bouillon and the plain bouillon yielded equally good precipitinogen. The precipitin titer of the sera was much lower than the agglutination titer but there was no precipitation in the controls and the results were quite definite. In Table II are given the results of cross-precipitation of the four strains employed for immunization of rabbits. There is precipitation of all the strains by each of the sera. Except in the case of Strain VIII the precipitinogen of each strain is precipitated best by the homologous serum. Serum VIII was rather a weak serum probably due to the fact that the rabbit from which it was obtained could not be injected regularly because of its having "snuffles." No great diversity among the strains is revealed by the precipitin titration. Strain IV, however, does seem to stand a little apart from the other three. Serum IV and Serum X were therefore selected for titration with all the strains. The results are recorded in Table III. The precipitinogens of all the strains except No. IV show greater precipitation with Serum X than with Serum IV. It appears that Strain IV is slightly different from the others immunologically as well as morphologically.

Pathogenicity for Rabbits.

According to Berger (1908), Holth (1908), and others, rabbits are the most susceptible of the small laboratory animals to experimental infection with *Bacillus pyogenes*. Guinea pigs are less susceptible, and mice least so.

In addition to the four rabbits used for immunization five others were injected intravenously with a single dose of 3 or 4 cc. of living bouillon culture, two with Strain IV and three with Strain X. Those immunized against Strains IV, VII, VIII, and X were repeatedly injected intravenously with these strains. Eight of the rabbits succumbed to the infection or were killed when in a badly crippled or moribund condition. The ninth may have died as a result of bleeding from the heart. There were no noticeable immediate symptoms following injections, no toxic symptoms, but usually a rise in temperature within 48 hours. Following this the rabbits appeared normal for 2 or 3 weeks. The first symptom of infection was often a progressive loss in weight followed by lameness or in four cases by paralysis. One of the paralyzed rabbits died during our absence from the laboratory and a complete autopsy was not obtained. The lesions found in the remaining seven rabbits may be classified as follows:

Lesions of bones in six rabbits (vertebræ three, femur four, rib one, tibia one).
Lesion of joint in one rabbit.
Lesions of muscles or tendons in three rabbits.
Lesion of lymph node in one rabbit.
Endocarditis in one rabbit.
Pneumonia in one rabbit.
Kidney abscesses in one rabbit.

All the above lesions were studied histologically and culturally, and were found to be due to the organism injected. A summary of these results is given in Table IV.

Rabbit J was injected subcutaneously only with bouillon cultures of Strain X at three different times as follows:

1st day. Injection A, 0.2 cc.; Injection B, 1.0 cc.

7th day. Injection C, 0.2 cc.; Injection D, 0.4 cc.; Injection E, 0.6 cc.

10th day. Injection F, 0.2 cc.; Injection G, 0.4 cc.; Injection H, 0.6 cc.

12th day. Rabbit chloroformed. Abscesses removed and fixed in Zenker's fluid. The rabbit had lost about 100 gm. in weight but was otherwise apparently well. At autopsy the subcutaneous lesions were the only ones found.

The abscesses at the time of removal were therefore 2, 5, and 11 days old. The youngest abscesses appeared grossly as flat discs about the size of five cent pieces and on gross section appeared to be composed of a fibrous tissue infiltrated with a small amount of yellowish

translucent viscid pus, and surrounded by soft hemorrhagic edematous tissue. The largest and one of the oldest abscesses, B, was firm and nodular, about 1 cm. in diameter, composed of a thick capsule of dense fibrous tissue enclosing a thick creamy yellow somewhat viscid pus.

A stained section of one of the youngest abscesses, H (Fig. 10), is roughly oval, about 7 mm. long by 2 mm. broad. It lies in a loose areolar connective tissue with a layer of transversely cut muscle fibers within 1 mm. of one side of the abscess. There is no definite capsule. The abscess is bordered by a thin layer of fibrin and necrotic connective tissue. The connective tissue on all sides is infiltrated by polymorphonuclear leucocytes while the perimysium of the adjacent muscular tissue contains many eosinophilic cells. There are also some large mononuclear cells many of which are doubtless fibroblasts though some may be endothelial leucocytes. Some of the muscle fibers nearest the abscess are invaded by polymorphonuclear leucocytes and are undergoing heterolysis. The contents of the abscess are principally a mass of degenerating polymorphonuclear leucocytes. Scattered about are bits of collagenic fibers, each embedded in a mass of *B. pyogenes* as though the bacilli are growing on the collagenic fibers as a medium (Fig. 14). Very few bacilli are found elsewhere than clustered about these fibers. About these individual masses of bacilli there is always an area of compact necrosed cells with few visible nuclei and in one part these areas by confluence have formed the beginning of the characteristic central zone of the older abscesses.

The older abscesses, A and B, are spherical and surrounded by dense fibrous capsules, 2 to 3 mm. thick (Fig. 11). The abscess is differentiated into three fairly distinct zones. The central zone (Fig. 11, *a*) is a ragged granular mass of dead and disintegrating cells with few visible nuclei. Within this zone many bacilli are scattered about, but towards its periphery these are in masses only a few of which still contain a fragment of collagenic fiber. Apparently these fibers are digested by the proteolytic action of the bacilli. Outside the central zone no bacilli are found. In the second or intermediate zone (Fig. 11, *b*) most of the cells have deeply stained nuclei which, however, exhibit pycnosis, caryorrhexis, or caryolysis. The outer zone (Fig. 11, *c*) resembles the central zone in general appearance. There are few visible nuclei, but no bacilli. It is bordered, however, by large mononuclear macrophages laden with nuclear and other cell debris.

In sections the bacilli are best stained by Gram's method used according to Holth's (1908) directions. Stirling's aniline gentian violet containing 1 or 2 per cent by weight of the dry stain has given us excellent results. To show the association of bacilli with collagenic fibers we have obtained excellent preparations with the following stains.

Orth's lithium-carmine, 20 minutes.

Acid alcohol, 3 minutes.

Stirling's aniline gentian violet, 10 seconds.

TABLE IV.

Rabbits Inoculated with B. pyogenes.

Rabbit.	Sex.	Strain No. Injection.	Maximum temperature.	Weight variation.	Localizations.	Result.
			°C.	gm.		
A	M.	IV. Repeated subcutaneous and intravenous injections for immunization.	40	1,890–2,355	Yellow spots in liver; hemorrhagic foci in lungs. (Incomplete autopsy.)	Died suddenly during night after being bled, 39th day after first injection of living culture.
B	"	VII. Repeated subcutaneous and intravenous injections for immunization.	41	1,650–1,865–1,380	Left femur; both kidneys; heart valve; epicardium.	Died after period of weakness 97th day after first injection of living culture.
C	"	VIII. Repeated subcutaneous and intravenous injections for immunization.	40.8	1,850–1,944–1,705	Muscle abscess in left quadriceps extensor femoris muscle. (Recurrent attacks of snuffles.)	Chloroformed 4½ mos. after first injection of living culture.
D	"	X. Repeated subcutaneous and intravenous injections for immunization.	40.2	1,650–2,010	3rd and 4th lumbar vertebræ; abscess surrounding and invading rib.	Paralysis of both hind legs. Chloroformed 5 mos. after first injection of living culture.
E	"	IV. 3 cc. intravenously.	39.8	1,960–2,125–1,945	(Incomplete autopsy.)	Paralysis of both hind legs and bladder. Died suddenly on 34th day after injection.

F	F.	X. 3 cc. intravenously.	41.2	1,935–1,980–1,040	Left femur.	Died 27 days after injection.
G	M.	IV. 4 " "	40.5	1,645–1,935–1,470	1st thoracic vertebra; right knee; both shoulder joints (periarticular abscesses).	Paralysis of left hind leg. Chloroformed on 81st day after injection.
H	"	X. 4 " "	41.4	1,835–1,895–1,240	Right femur; tendon of left gastrocnemius muscle; left instep; lungs.	Died on 13th day after injection.
I	"	X. 3 " "	39.8	1,815–1,910–1,240	1st, 2nd, and 3rd lumbar vertebræ; crest of right tibia; trochanter and gluteus minimus muscle of right femur; right zygomatic fossa; right biceps brachii muscle; left cubital gland.	Paralysis of both hind legs. Chloroformed on 64th day after injection.
J	"	X. Multiple subcutaneous injections.	39.4	3,580–3,415	Subcutaneous abscesses at points of injection.	Chloroformed 11th day after first injection.

Wash in water.
Lugol's iodine solution, 30 seconds.
Wash in water. Blot.
Decolorize in absolute alcohol.
Wash in water.
Mallory's aniline blue and orange G, 20 minutes.
Wash in water.
Decolorize and dehydrate in 95 per cent and absolute alcohol.
Xylol. Mount in balsam.

By the above method *B. pyogenes* is stained purple, collagenic fibers bright blue, leucocytes and tissue cell nuclei red or deep orange, blood, fibrin, and muscle yellow.

Of the lesions resulting from the intravenous injection of cultures a few deserve brief description. The most frequently produced lesions were those of the bones. Such lesions have been reported by Berger (1908), Holth (1908), and Koske (1906). Their protocols show that they encountered paralyses also in experimentally infected animals. Koske, unable to find lesions in the cords of paralyzed pigs, considered it possible that a specific neurotoxin might be involved but was unable to obtain toxic effects with filtered cultures. He does not mention examining the vertebræ of these animals. Four times we encountered paralyses in injected rabbits. In the three that were thoroughly autopsied were found lesions on the ventral floor of the spinal canal. These lesions were not visible from the ventral side of the spinal column and in fact could not be found until the cord had been removed. There were then found abscesses exerting pressure against the ventral side of the cord. In no case was the dura penetrated nor were the meninges infected. In the case of Rabbit G an abscess lay between the dura and the vertebral periosteum without invading the body of the vertebra. In Rabbits D and I were found intervertebral abscesses obliterating the intervertebral cartilages, invading the bodies of the vertebræ, and eroding the bone with more or less destruction of the floor of the spinal canal (Fig. 12).

In a stained section there is found proliferation of connective tissue about the abscess. The center of the focus is composed of a mass of disintegrated cells and nuclei which appear to have been small mononuclear cells rather than polymorphonuclear leucocytes, differing in this respect from the abscesses in the subcutis and other soft parts. In the proliferating fibrous tissue bordering and sometimes sur-

rounding the abscesses in the vertebræ are many large mononuclear cells (Fig. 15). Stained by Gram's method many of these large cells are seen to be filled with *B. pyogenes* (Fig. 16). Holth regards these cells as young fibroblasts rather than endothelial cells. In the necrotic center of the abscess bacilli lie scattered about.

The lesion here described is much like the "*grösseren Knoten*" described by Holth and found by him in various parts of the body—lungs, subcutis, peritoneum, etc. He describes masses of "*Rundzellen*" in the center of the abscess undergoing necrosis and disintegration, an intermediate zone of tissue resembling the round cell masses of smaller abscesses, and an outer capsule of connective tissue interrupted by small masses of round cells. As the tumor grows the round cell masses fuse and the process of disintegration advances. He then describes the bacilli scattered about in the center of the abscess and within what he regards as large connective tissue cells of the capsule. He does not mention the presence of polymorphonuclear leucocytes in the abscess, whereas we found them to predominate in abscesses of soft parts. It is to be noted, however, that because of pycnosis these cells often resemble round cells. It is to be noted also that in the subcutaneous abscesses described above no bacilli were found in the cells of the capsule, possibly because the abscesses studied by us were not old enough.

Lesions in the long bones—femur and tibia (Fig. 13)—were fundamentally like those in the bodies of the vertebræ, modified by the tissues encountered. The predominating cells of the reaction resembled plasma cells. In places there was marked proliferation of connective tissue. In places the bacilli appeared to grow freely among the cells of the bone marrow. Occasionally bone lacunæ were seen filled with masses of bacilli. There was always erosion of the bone making it quite porous. In two cases the femur broke under very little strain as the rabbits were being tied out for autopsy. A nodular encapsulated abscess enveloped the rib of Rabbit D eroding it from without. The abscess may have been subperiosteal in origin.

The knee of Rabbit G contained a glairy viscid pus and one of the joint surfaces was eroded.

In Rabbits C, H, and I true myositis and tendinitis were encountered. In these cases the muscle fibers had been completely heterolyzed or digested and the muscle converted into a closed sac of viscid

glairy pus within the epimysium (Fig. 9). There were present in the pus and perimysium plasma cells, endothelial leucocytes, neutrophilic polymorphonuclear leucocytes, and, especially conspicuous, eosinophilic leucocytes. Bacilli were abundant in all but the oldest lesions.

The diseased mitral valve of Rabbit B was encrusted with masses of *Bacillus pyogenes*, leucocytes, and necrotic tissue. The tissue of the valve was largely fibrin.

In the pneumonic lung of Rabbit H masses of bacilli were found beneath the pulmonary pleura. It appeared that growth had started from bacilli lodged in the pleural capillaries. The alveoli were filled with blood, fibrin, and desquamated epithelium.

The kidney abscesses of Rabbit B were in the form of pyramids with bases at the cortex. Around the borders of the abscesses the glomeruli and the capillaries of the interstitial tissue were plugged with leucocytes and masses of bacilli. The interlobular arteries were similarly plugged.

Resemblance to Other Organisms. Classification.

The close resemblance of *Bacillus pyogenes* to streptococci has been mentioned above. This is especially true of some strains when grown on certain media, a resemblance so close that the bacteriologist working with milk or animal diseases must be on his guard not to confuse them. *Bacillus pyogenes* produces laking of a suspension of blood or blood corpuscles in salt solution as do the hemolytic streptococci of human origin (Brown, 1920). Its limiting hydrogen ion concentration in dextrose bouillon would also mislead one to place it among the human streptococci (Avery and Cullen, 1919). On the other hand, *Bacillus pyogenes* liquefies coagulated blood serum and is distinctly diphtheroid at times. Morphologically, therefore, it may be one of the diphthero-streptococci now and then described. Such organisms, are occasionally isolated from human lesions, especially pneumonias and though *Bacillus pyogenes* has never been identified in man, it would be well for bacteriologists to keep it in mind. Since it is found in several species of animals and is widely disseminated in milk it may be that there are rare cases of human infection.

There is also found in animals a group of small Gram-positive organisms closely resembling *Bacillus pyogenes* morphologically and culturally. We have studied two such strains from the livers of calves and one from the lung of a hog. The strains from calves were apparently alike, and had the same fermentation reactions as *Bacillus pyogenes*. They were cultivated with greater difficulty than the latter, however. They grew best under partially anaerobic conditions and would not grow in serum-free media. There was no apparent growth in milk. Gelatin and coagulated serum were not liquefied and growth on the latter was scarcely visible. There was very little laking of horse blood in agar plates. A rabbit was repeatedly injected intravenously with several cubic centimeters of serum bouillon cultures of one of these strains with no ill effects. Serum from this rabbit precipitated precipitinogens of both of these strains in dilution of 1:80 or 1:160 but produced no precipitation of the precipitinogens of strains of *Bacillus pyogenes*. Neither did *Bacillus pyogenes* antisera precipitate precipitinogens of these two strains.

A strain from a hog also resembled *Bacillus pyogenes* morphologically. It differed from *Bacillus pyogenes* and the other two strains just described in that it fermented salicin. It resembled the two strains from calves in failing to liquefy gelatin or coagulated serum but grew much better than those strains. No visible change was produced in milk but sufficient acid was produced so that when the culture tube was placed in boiling water the milk was coagulated. An indefinite zone of hemolysis was produced in the blood agar plate.

Glage (1913) points out a certain resemblance of *Bacillus pyogenes* to the bacillus of swine erysipelas. Morphology, growth in gelatin, and the lesions produced in hogs and small experimental animals serve to differentiate the two.

Dunkel (1908) thought that *Bacillus pyogenes* could be transformed into *Bacillus pseudotuberculosis ovis* by animal passage and therefore regarded the two as of the same species. The two are certainly not alike when studied as isolated from their respective hosts. The dry colonies described as characteristic of *Bacillus pseudotuberculosis*, pigmented colonies on coagulated blood serum, and failure to produce change in milk are not at all like *Bacillus pyogenes*. There may be some morphological resemblance.

Priewe (1911) and Glage (1913) have claimed that *Bacillus pyogenes* belongs to the influenza bacillus group for the following reasons: hemoglobinophilic habit, form, size, non-motility, lack of spores, abundance in green pus, growth at high temperature only, and slight virulence for small laboratory animals. Priewe claimed that a *Bacillus pyogenes* antiserum agglutinated *Bacillus influenzæ*. We have not tried to repeat the latter observation. However, we do regard it as well established that *Bacillus pyogenes* is Gram-positive and that it is not hemoglobinophilic. The other characters enumerated by the above authors are common to so many and dissimilar organisms that they are of little value as evidence of relationship of these two organisms. *Bacillus pyogenes* does not have the foul odor characteristic of *Bacillus influenzæ* and the two do not produce similar lesions in rabbits. Grips (1903), Grips, Glage, and Nieberle (1904), and Priewe (1911) have regarded swine-plague as due primarily to *Bacillus pyogenes* with *Bacillus suisepticus* as a secondary invader. They regard the primary infection as a "*Tierinfluenza*." The views of these authors have not been accepted by others. Olt (1904) has criticized them thoroughly but asserts that many of the lesions characteristic of chronic swine-plague are due to *Bacillus pyogenes*.

The forms assumed by *Bacillus pyogenes* bear a striking resemblance to those of *Asterococcus mycoides* described by Borrel, Dujardin-Beaumetz, Jeantet, and Jouan (1910) as the cause of bovine pleuropneumonia. The latter organism is, however, much the smaller. The work of the authors mentioned is almost wholly morphological and the result of staining by Gram's method is not mentioned. Buchanan (1918), however, places *Asterococcus* in the Gram-negative subtribe Hemophilinæ. The other genus of this subtribe is *Hemophilus*, of which the type species is *Hemophilus influenzæ*, the influenza bacillus of Pfeiffer. Since Buchanan and Murray (1916) describe *Bacillus pyogenes* as a member of the hemophilic or influenza group they presumably classify it also in the subtribe Hemophilinæ where it can hardly belong in view of its being Gram-positive. Preisz (1906) regarded *Bacillus pyogenes* as one of the "*Corynebakterien*." We are more inclined to place it in the genus *Corynebacterium* as defined in the Preliminary Report of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types (1917) than in the so called influenza group.

SUMMARY.

Bacillus pyogenes is probably quite common in this country, as it is known to be in Europe.

A careful study of twelve strains from cattle and one from a hog has disclosed the following characteristics which have not been reported or have been in dispute.

Bacillus pyogenes is Gram-positive and pleomorphic, producing forms ranging from short chains of streptococcoid elements to branching filaments.

It is hemolytic, producing the beta type of hemolysis in blood agar. It is not hemoglobinophilic, though its growth is greatly favored by some higher protein material such as egg albumin, serum, or blood.

It ferments xylose in addition to the substances previously reported.

The coagulation of milk by *Bacillus pyogenes* is primarily an enzyme coagulation and the subsequent digestion of the curd takes place in an acid medium.

The intravenous injection of rabbits was invariably fatal. The lesions most commonly developed were those of the bones. Paralysis was frequently produced, and in each case was caused by lesions in the vertebræ exerting pressure against the ventral columns of the spinal cord. Muscle abscesses were also frequently produced.

The authors regard the organism as belonging to the *Corynebacteria* rather than to the influenza group.

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EXPLANATION OF PLATES.

PLATE 14.

FIG. 1. A horse blood agar plate culture of *B. pyogenes* X after incubation for 48 hours. The large round hemolyzed area was produced by a few particles of saponin.

PLATE 15.

Gram stains. Magnification × 1,000.

FIG. 2. The bacillary form of Strain X.

FIG. 3. The fusiform form of Strain IV.

FIG. 4. The fusiform form of Strain X.

FIG. 5. The filamentous or branching form of Strain IV.

FIG. 6. The diphtheroid form of Strain X showing a few buds.

FIG. 7. The streptococcoid form of Strain IV.

FIG. 8. The streptococcoid form of Strain X.

PLATE 16.

FIG. 9. Rabbit H. Abscess in tendinous end of gastrocnemius muscle at *a*. Above the abscess is a section of the distal end of the flexor digitalis pedis sublimis muscle. Eosin and methylene blue stain. × 10.

FIG. 10. Rabbit J. Subcutaneous abscess H, 48 hours after injection. The capsule and zonal arrangement of the abscess not yet developed. Gram and aniline blue stain. × 5.

FIG. 11. Rabbit J. Subcutaneous abscess A, 11 days after injection. A very thick fibrous capsule (*d*) about the abscess and the three zones (*a*, *b*, *c*) of the abscess described in the text are shown. Eosin and methylene blue stain. × 5.

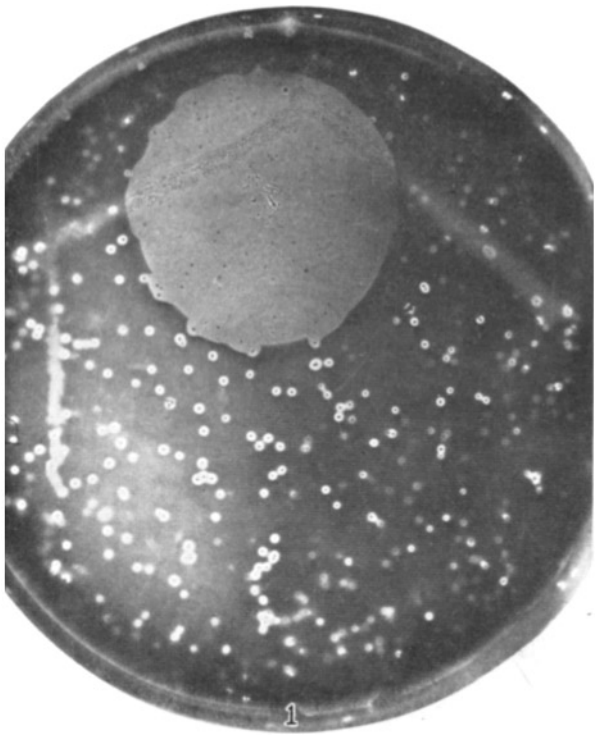
FIG. 12. Rabbit D. Abscess occupying the body of the third lumbar vertebra at *a*, the spinous process and dorsum of the spinal foramen having been removed. Only a small bony fragment of the floor of the spinal canal remains at *c*. Eosin and methylene blue stain. × 5.

FIG. 13. Rabbit I. Abscess in crest of tibia at *a*. Transverse section. Eosin and methylene blue stain. × 10.

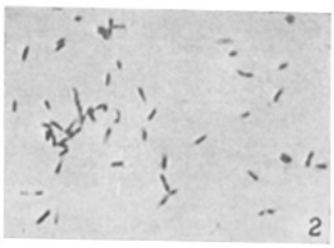
FIG. 14. Rabbit J. A field from the center of the abscess shown in Fig. 10, showing the minute bacilli clustered about remnants of connective tissue fibers. Gram and aniline blue stain. × 430.

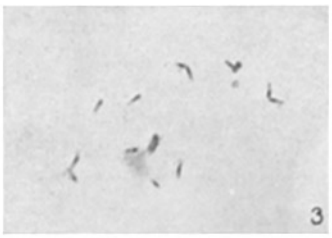
FIG. 15. Rabbit D. A field from the region marked *b* in Fig. 12, showing large mononuclear cells probably on the border of an abscess. Eosin and methylene blue stain. × 430.

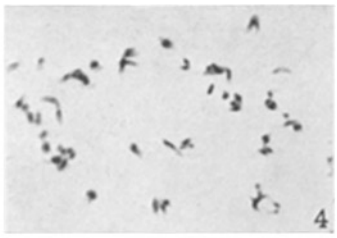
FIG. 16. Rabbit D. A field from the same region as Fig. 15, showing the large mononuclear cells filled with *B. pyogenes*. Gram stain. × 430.

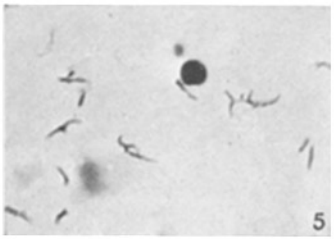


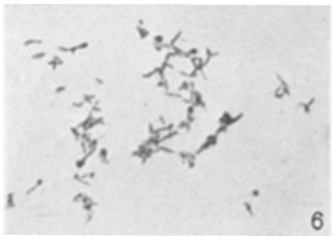
(Brown and Orcutt: *Bacillus pyogenes*.)

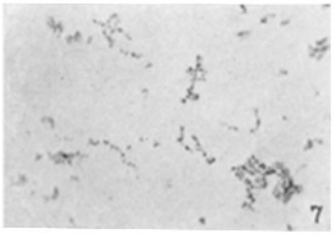


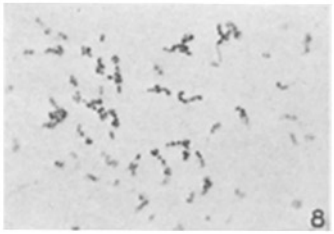




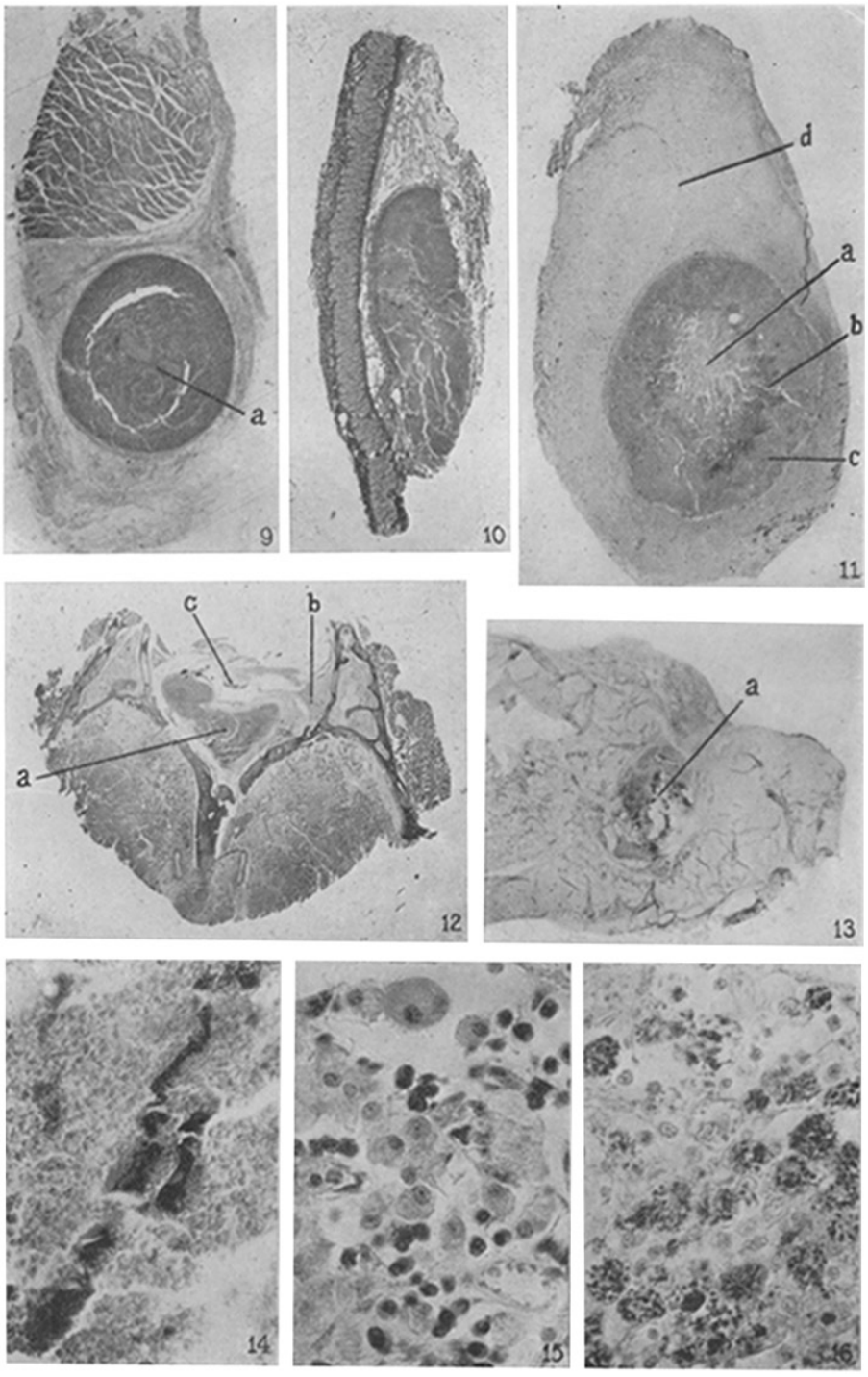








(Brown and Orcutt: *Bacillus pyogenes*.)



(Brown and Orcutt: *Bacillus pyogenes*.)