

A CASE OF ACUTE ENDOCARDITIS CAUSED BY MICRO-  
COCCUS ZYMOGENES (NOV. SPEC.), WITH A DE-  
SCRIPTION OF THE MICROORGANISM.\*

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*Clinical History.* September 14, 1898, a German, aged 37 years, was admitted to the service of Dr. Osler, complaining of fever which had persisted since early in July. The history of the patient's family and his occupation were of no importance. In 1889 the patient was confined to bed three weeks with "rheumatic fever," though no joint symptoms were recalled. Otherwise the history was that of a healthy man of good habits.

July 4, a severe cold was contracted, fever appearing on the 6th and persisting until early in August when it subsided but reappeared about the middle of the same month, and for this relapse in supposed typhoid fever the patient was sent to the hospital. Severe frontal headache, stiffness and pain in the right shoulder joint, epistaxis, loss of strength, anæmia, and loss of 26 pounds in weight were noted during July and August.

Examination on admission showed a well-built, emaciated man, with a moderate grade of anæmia, and a temperature of 103° F. There was decrease in the area of cardiac dulness, and a pure diastolic murmur was detected over the body and base of the heart, suggesting disease of the aortic valves. The pulse also indicated incompetency of these valves. Over the abdomen were scattered a few erythematous spots thought at the time to be the rose spots of typhoid fever. The spleen was large, readily palpable and rather firm. The right shoulder joint which in July had been painful and stiff was now apparently normal. The urine showed a well-marked Ehrlich's diazo-reaction and a faint trace of albumin. During the first ten days in the hospital, anæmia, emaciation, fever, enlarged spleen and the cardiac condition of aortic incompetency

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were the most striking symptoms. At times stiffness and pain about the right shoulder joint were complained of, but there were at no time signs of inflammation about the joint.

The high temperature of 103-104° F. and a leucocytosis of 18,000 persisted. A suggestive Widal reaction also was present.

Though in a satisfactory condition on the evening of September 23, the next morning the patient appeared much more seriously ill. Delirium of the night previous had given way to stupor; anæmia was of extreme grade; the extremities were cold; and the appearance one of collapse. The lungs were quite clear but the heart, dilated to twice its previous size, had become rapid and irregular in action, and the physical signs of aortic disease were no longer recognizable. On the afternoon of September 23, the maximum cardiac impulse was readily located in the 5th left interspace, 9 cm. from the midsternal line, and the area of relative and absolute cardiac dulness was less than normal; on the morning of the 24th the maximum cardiac impulse was in the 6th left interspace 12.5 cm. from the midsternal line and the area of relative and absolute cardiac dulness had increased to twice that of the normal. During the night of September 24, the cardiac condition had somewhat improved and endocardial murmurs and a palpable thrill appeared at the apex suggesting both mitral and aortic disease.

At this time agar plate cultures from the blood were prepared on which within 48 hours grew colonies of what appeared to be a short-chained streptococcus, which subsequently on various media proved to be a micrococcus occurring mostly in pairs.

There subsequently occurred repeated attacks of collapse from cardiac failure up to the time of the patient's death on October 3, 1898.

October 1, three days before death, blood cultures were again prepared. On these cultures there grew the same micrococcus occurring mostly in pairs, the growths on various media corresponding to those obtained from the cultures prepared September 24.

*Autopsy.* Performed on the day of death, the body having been kept on ice and showing no evidences of post-mortem change. Description will here be given of only those organs which were the seat of pathological change.

*Anatomical diagnosis.* Subacute and acute ulcerative endocarditis of aortic and mitral valves; acute splenic tumor; septic infarctions in spleen and kidneys; embolic abscess in intestinal wall; bronchopneumonia; chronic diffuse nephritis of moderate degree.

Body emaciated, 184 cm. long; rigor mortis slight. Abdominal muscles of a deep red color; no excess of peritoneal fluid; peritoneal surfaces

smooth and glistening; a few old pleuritic adhesions; pericardial layers smooth.

*Heart* distended with liquid blood; weight 420 grammes. Nothing abnormal in the right heart. On the left side the mitral valve showed slight old thickening and in the middle of the edge of the aortic segment was a small, dark-red, fresh vegetation; many of the chordæ tendinæ had been ruptured, the ends of the ruptured cords being coated with fibrinous deposits; others were markedly thinned in the median portion, the adjoining parts being much thickened by fresh vegetations. The aortic valves were matted together by the exuberant vegetations which had formed upon them; a prolongation on the anterior coronary segment formed a flattened plate, which was apparently in part at least a mass of organized tissue, perforated by a hole about 2 mm. in diameter; the posterior coronary segment was surmounted by a rough mass of vegetations; the mitral segment was similarly covered with vegetations through which a ragged perforation was evident. From a point on the ventricular wall at the base of the posterior coronary segment to the base of the aortic segment of the mitral valve there ran a cord, like a moderator band, thickened in its median portion by beaded vegetations and entangled in the clots formed about the aortic vegetations. There were fresh vegetations also on the ventricular wall and on the wall of the aorta just above the valves.

*Spleen*, bound up in adhesions with the diaphragm and parietal abdominal wall, was much enlarged and very soft so that on attempting to remove it the capsule was ruptured in several places. Weight 470 grammes. The surface was peculiarly discolored over irregular areas which occupied a large portion of the capsule. Beneath some of these the substance was firmer than normal and on section elevated above the surrounding tissue, such areas corresponding to firm yellowish-white masses extending into the splenic pulp and sharply marked off by hæmorrhagic zones. All degrees of softening of such masses were present and the larger discolored portions of the capsule proved to form merely thin walls covering irregular cavities in the spleen filled with purplish-brown grumous fluid. These cavities also were delimited from the surrounding tissue by mottled zones of red and white. The relatively normal splenic pulp between the areas of infarction was much swollen and the Malpighian bodies and trabeculæ could be made out only with great difficulty.

*Kidneys* also presented superficially areas of purplish and grayish discoloration, of varying form and size, sharply demarcated from the surrounding cortex by lines of congestion and hæmorrhage. On section

these areas were seen to correspond with somewhat wedge-shaped masses varying in consistence and color. The smallest and evidently the freshest of these were firmer than normal, yellowish-white and elevated above the surface of the adjacent tissue; the larger and older masses were much softened and of a grayish-purple color throughout. In addition, the kidneys presented evidences of moderate chronic diffuse nephritis.

In the lower third of the *ileum* one of the lymphatic nodules in the mucosa appeared much swollen and reddened with a zone of hæmorrhage round about it. This nodule was quite firm and elevated several millimetres above the surface of the mucosa.

The left *lung* showed small bronchopneumonic patches throughout the lower lobe.

*Microscopic examination.* Sections of the diseased organs showed both acute and subacute morbid processes. Sections of the cardiac valves and vegetations showed a considerable curling and twisting of the valve proper. Thrombus masses surmounted the valves and formed the support for a fairly advanced growth of granulation tissue. The more superficial portions of the vegetations, however, consisted of platelets, fibrin and leucocytes, together with myriads of micrococci which, stained by Weigert's or Gram's method, formed dense bluish-black masses throughout the thrombus. Smear preparations from the vegetations showed the micrococci apparently unmixed with any other organisms and occurring often within leucocytes.

Sections through the discolored and softened areas in the kidney showed the typical picture of septic infarction with complete necrosis of the epithelium and the invasion of many polymorphonuclear leucocytes. The blood-vessels near the apex of the wedge were in many instances plugged with masses of micrococci. The firmer whitish masses both in the kidney and spleen were simple anæmic infarctions.

The small nodule in the *ileum* showed on section a mass of lymphatic tissue in the centre of which a small artery was completely occluded by a dense hyaline thrombus; about the vessel was an accumulation of polymorphonuclear leucocytes and nuclear fragments, together with a considerable exudation of fibrin; the neighboring vessels were also congested.

*Cultures.* Agar cultures in Petri dishes were made from the heart's blood, valvular vegetations, gall-bladder, splenic and renal infarctions, and other parts, and in all of these there appeared numerous, minute, pin-point, somewhat opaque, white colonies in the depths of the medium, and corresponding superficial colonies. The latter were also small, rarely exceeding the size of a pin's head. They were round in outline,

smooth, glistening, transparent, almost colorless or, on microscopical examination, perhaps slightly brownish, and slightly elevated above the surface of the medium. No other kind of colonies appeared on the Petri plates.

From the deep and superficial colonies obtained from the different organs a single species of microorganism was isolated and studied in all of its essential characters.

#### DESCRIPTION OF THE MICROORGANISM.

*Morphology.* Coverslips prepared from the original colonies and from the secondary tube-cultures showed a micrococcus in every way identical morphologically with that found in smears from the cardiac valves and with that isolated from the blood during life.

The organism is an extremely minute micrococcus, often somewhat elongated or elliptical in outline. It is much smaller than the *Micrococcus lanceolatus*. It occurs often singly, often in masses, but by far most frequently in pairs, two or more pairs being sometimes united into short chains, and indeed in cultures made in a hanging drop of bouillon chains of twenty or more members may occasionally occur, although in such a drop the usual grouping is in pairs. In the pairs or short chains the longer axis of the coccus is often transverse to that of the chain.

No motility is to be observed in the hanging drop other than the oscillatory motion common to all fine particles in suspension.

The organism stains well with the ordinary aniline dyes and by Gram's method remains deeply stained. No evidence of the presence of a capsule has been obtained either in cultures or in the tissues.

*Cultural characteristics: Agar.* Smears made in slanted agar tubes give a rather profuse, thin, slightly elevated growth along the line of the smear. The growth is somewhat moist and glistening, almost colorless; by reflected light, pale grayish-white; by transmitted light, transparent and of a somewhat smoky or brownish tint, old cultures showing this smoky discoloration more distinctly. The growth is not always, however, a diffuse flat expanse, but is often made up of a conglomeration of minute dew-drop-like colonies. The margins are somewhat crenated by the presence of larger, and sometimes

discrete, glistening colonies, slightly more elevated than those nearer the centre. Superficial, discrete colonies on agar may somewhat exceed one millimetre in diameter.

*Glycerine agar.* Cultures on glycerine agar grow more profusely but in other respects are identical with those on plain agar.

*Ascitic-fluid agar.* On ascitic-fluid agar the growth is distinctly more profuse and opaque than upon plain nutrient agar.

*Glucose agar.* The growth along the stab in glucose agar is moderately profuse but neither in stab cultures nor in cultures made in liquefied glucose agar, which is then allowed to solidify, is there any production of gas.

*Bouillon.* Broth becomes slightly clouded after twenty-four hours' growth, but in the course of three or four days the organisms settle to the bottom and form a whitish sediment leaving the overlying fluid clear. No indol is produced.

*Sugar bouillon.* In glucose and in lactose litmus bouillon (Smith's) in Smith's fermentation tubes acid is produced without gas, the medium becoming turbid in both arms and decolorized in the closed arm.

*Potato.* On potato, minute colonies appear and in about 36 hours become elevated, moist, of a somewhat dirty white color and pasty consistence, and after 72 hours confluent. Later the growth becomes rather dry and brownish. Occasionally there is no visible growth on potato.

*Gelatine.* In stab cultures in gelatine a somewhat opaque, granular growth extends along the line of stab and after about 36 hours a slight cupping appears at the surface and extends slowly downward, producing a tubular area of liquefaction which gradually involves the whole medium. Liquefaction is slower than in the case of *Staphylococcus pyogenes aureus*.

In gelatine plates the organism forms small pale granular colonies, yellowish by transmitted light, which float in small areas of liquefied gelatine.

*Blood serum.* On coagulated blood serum the growth is profuse and similar to that on agar, although perhaps somewhat less trans-

parent; along the line of smear the medium becomes somewhat translucent and is depressed, forming a definite groove below the surrounding surface. This change is similar to that observed in cultivations of *Bacillus subtilis* on blood serum. This partial liquefaction is accompanied, as a rule, with the production of a clear fluid and the breaking down of the solid medium.

*Milk.* The most characteristic cultural features of this organism appear in milk. In litmus milk of neutral reaction the immediate result of the growth is decolorization of the litmus within a period of four hours, a thin blue layer remaining at the surface. This decolorization is evidently due to deoxidation as the color returns to a certain extent on shaking the fluid with air, the returning color being, however, distinctly nearer to red than the original. Within twenty-four hours the milk becomes quite firmly coagulated, with the bluish or reddish layer still at the surface and the absence of color below. Next in time the upper layers of the coagulum become translucent and are gradually changed into a somewhat turbid fluid which is definitely red in the more superficial part and yellowish below. This softening and liquefaction of the coagulum progresses day by day, the layer of red fluid increasing in depth and the coagulum being transformed into a flocculent granular material floating in a yellowish liquid; finally, after several days, the whole coagulum is transformed and the red color extending into the depths stains the precipitate throughout; settling of this precipitate to the bottom of the tube at last leaves a clear straw-colored supernatant fluid overlying a dark red sediment.

These changes produced in litmus milk are absolutely constant and serve to distinguish this micrococcus sharply from related members of the pyogenic group of cocci.

*Production of enzymes.* Attempts were made to determine the nature of some of the metabolic products of the micrococcus.

Grown in sugar-free bouillon the reaction of the culture is alkaline, whereas in ordinary sugar-containing and lactose-containing bouillon it is acid. The acid reaction of milk cultures is, therefore, doubtless due to the production of acid by the decomposition of the milk sugar.

Experiments were made to determine whether in addition to the

formation of acid a milk-curdling ferment (Labferment, rennin, pexin) was produced in sufficient quantity to coagulate milk. Milk cultures, which had reached the stage of coagulation, were passed through a Pasteur filter to remove the bacteria and the resulting clear filtrate was added in small quantity to sterilized litmus milk. No appreciable change in the reaction of the milk followed this addition, but within 36 hours there was produced a coagulum quite as firm as that caused by the action of the living micrococci themselves. No decolorization nor acidification of the medium occurred. In the course of time the coagulum retracted somewhat, leaving a clear fluid overlying it. This experiment establishes the existence of a rennin-like ferment among the products of the micrococcus. The solution of the coagulated casein, as well as the liquefaction of gelatin and blood serum, indicate the formation of a proteolytic ferment related more or less closely to enzymes concerned in the digestion of proteids. The sterile filtrate, obtained by passing through a Pasteur filter milk cultures in which the liquefaction of the coagulum is in progress, was added to sterile milk and to nutrient gelatine for the purpose of observing the production of these changes without the presence of the organisms themselves. In these experiments the curdling of the milk proceeded as described above without much change in the reaction. On standing in the thermostat, this coagulum has of course a tendency to contract, but a further diminution in its size by the softening and liquefaction of its superficial layers is quite evident. The liquefaction is, however, not so complete as when the living organisms are present.

When a few drops of the filtrate are dropped upon the surface of sterile nutrient gelatine, this medium becomes slowly liquefied with the production of a clear fluid. Control inoculations in bouillon, milk and agar from these tubes of coagulated and peptonized milk and of liquefied gelatine, gave uniformly negative results, and coverslips from them showed no organisms.

The conclusion, therefore, is justified that this micrococcus produces both milk-curdling and proteolytic enzymes, separable from the bacterial cells and capable, when thus isolated, of producing their characteristic effects in milk and in gelatine.



*Relation to oxygen.* The micrococcus is a facultative anaërobe, the cultures grown in Buchner jars presenting quite as luxuriant a growth as those kept in the open air. Grown in hydrogen in a Novy jar, however, the colonies are rather more scanty and thinner.

*Vitality.* The organism is very hardy and tenacious of life. Agar cultures which stood in the laboratory from October until February and which were very much dried out furnished a profuse growth on a fresh agar tube. In this respect it obviously stands in marked contrast to *Micrococcus lanceolatus* which can be kept alive only with much difficulty.

Moderate variations in temperature seem to have no deleterious influence on the micrococcus. Indeed, it can withstand a temperature higher than that which is fatal to many micrococci. In order to fix the thermal death-point, portions of a bouillon suspension of the organism were sealed in Sternberg bulbs and heated for a period of five minutes each, to different temperatures. After exposure for five minutes to a temperature of 65° C., no organisms grew when the bouillon was transferred to the surface of an agar slant. After heating to 62°, only one or two colonies developed, but exposure to a temperature of 60° for five minutes produced hardly any retarding effect upon the growth of the organisms.

To antiseptics, such as carbolic acid and chloroform water, the micrococcus is considerably resistant, a relatively large percentage of antiseptic being required to kill all of the organisms in bouillon cultures.

*Pathogenesis.* The ordinary laboratory animals were inoculated in various ways with cultures and suspensions of the organism.

*White mice.* These animals, inoculated intraperitoneally with various quantities of thin suspensions of the organisms in bouillon, died in the majority of the experiments after periods varying from seven hours to three or four days, with evidences of general infection. Autopsy showed always a much swollen spleen and sometimes reddened lymph glands. The micrococci were recovered in pure culture from the heart, spleen, liver, and kidney. The dose given varied from 0.3 to 0.7 cc. of the suspension.

White mice were inoculated with a loopful of the solid culture also subcutaneously, but although they died after several days, the organism was not recovered from the organs. Inoculated subcutaneously with relatively large quantities of the bouillon suspension (0.8 to 1.8 cc.) they died within four to ten days, the organs showing the same changes as after intraperitoneal inoculation and yielding the cocci again in pure culture. No macroscopic lesion of the muscles near the seat of subcutaneous inoculation was visible.

*Wild mice* proved to be distinctly less susceptible than the white, although occasionally they succumbed to intraperitoneal inoculation with general septicæmia.

*Guinea-pigs, white rats and pigeons* showed very little susceptibility to inoculation.

*Rabbits.* A number of rabbits were inoculated, for the most part intravenously, but also intrapleurally and intraperitoneally. The latter modes of inoculation were followed by recovery of the animal. Indeed, rabbits appear, in general, rather resistant to this organism, since of eight rabbits inoculated only one developed a general septicæmia. Two of the rabbits showed at the site of inoculation at the time of death, abscesses which contained the micrococcus, and from one of them, as well as from the animal which died with a general infection, the organism was recovered from the contents of the distended urinary bladder.

One of the rabbits received 2.5 cc. of a thick bouillon suspension, containing particles of sterilized potato, into the ear vein. This rabbit became very ill and died after five and a half days, the micrococcus being recovered in pure culture from the blood and all the organs. The condition of the heart was most interesting. The mitral valve showed the presence of two quite firm vegetations, one on each leaflet, that on the aortic leaflet being the larger and measuring about 2 mm. in diameter. The thrombus mass was situated very near the edge of the valve and there was a propagated dark red clot running upward toward the base of the valve on the auricular surface. The smaller vegetation on the opposite leaflet of the mitral valve was situated somewhat nearer the base of the valve, and also on the

auricular surface. The papillary muscles appeared opaque and yellowish. The other valves, as well as the musculature, were apparently normal. Smears from the mitral vegetations showed the presence of the micrococci, unmixed with other organisms, and cultures from these vegetations gave a pure growth of the micrococcus.

Thus of seven rabbits inoculated three showed no ill effects, two died after several days with no apparent lesions at autopsy and no evidence of general infection (from these the organism was not recovered), one died of an intercurrent affection with a small abscess containing the micrococcus at the point of inoculation, one died with a large abscess at site of inoculation, the organism being recovered from the abscess and the distended urinary bladder, and finally one rabbit died with a general septicæmia, together with an *acute vegetative mitral endocarditis*, from which the organism was recovered in pure culture.

*Dogs.* Two dogs were also inoculated. One of these showed no ill effects, although 10 cc. of a thick suspension, containing particles of potato, were injected into the jugular vein; after sixteen days this animal was killed but at the autopsy the organs appeared quite normal and cultures from all parts were sterile.

In the case of the second dog, Rosenbach's procedure was adopted. The left carotid artery was opened under aseptic precautions, the aortic valves were torn through and lacerated by means of a long probe passed down the carotid, and the artery was then ligated on both sides of the opening. Two cubic centimetres of a thick suspension of the micrococcus in bouillon were then injected into the left jugular vein which was then ligated and the wound of the skin was closed with sutures. The dog appeared rather ill for a few days, but evidently improved slightly after the fourth day. Immediately after the operation there was detected a loud and distinctly diastolic murmur which persisted until the seventh day when the dog was killed with chloroform and an autopsy performed.

The spleen was found enlarged, congested and partly covered with a fibrino-purulent exudate. The other organs were macroscopically apparently normal with the exception of the heart. The right side of

the heart was normal. The aortic valves were the seat of an *acute and subacute endocarditis*. The probe had passed into the sinuses of Valsalva and had penetrated into the muscular tissue of the septum ventriculorum, running down 1.5 cm. just to, but not piercing, the endocardium over the septum. On section the muscle about this area was grayish and translucent in appearance with grayish opaque dots scattered over the translucent area. A second injury had occurred in the sinus behind the mitral segment of the aortic valve, the probe perforating the base of the valvular segment and passing into the ventricular cavity near the base of the mitral valve. About this opening there were on both surfaces of the valve granular translucent vegetations which extended upon the mitral valve and also upon the intima of the aorta just above the aortic valves. Coverslips from the vegetations and necrotic muscle showed numerous micrococci in pairs, many of them within leucocytes. Cultures from the same locations gave a pure growth of the micrococcus, as did cultures from the blood, liver, spleen, and kidney.

*Toxine.* Attempts were made to obtain a toxine from cultures of this organism, but so far with little success. A priori, it would not be expected that an organism so closely resembling in its general properties the pyogenic group of cocci would produce a toxine of high virulence. The wide dissemination of the cocci observed in the cases of general infection would also point to the same inference, and this suggestion is supported by the few experiments thus far made relating to this point.

A culture, made in a large quantity of sugar-free bouillon and allowed to grow in a Roux flask for three weeks, was killed with chloroform. Ten cubic centimetres of this killed culture, after evaporation of the chloroform, were injected into the ear vein of a rabbit, a control rabbit receiving a similar injection of the same quantity of sterile sugar-free bouillon. Neither rabbit showed elevation of temperature or any other ill effect following the injection.

#### SUMMARY.

From a case of acute endocarditis of the aortic and mitral valves with infarctions in the spleen and kidneys a micrococcus was twice

isolated in pure culture from the blood during life and was demonstrated after death both microscopically and in pure culture in large numbers in the valvular vegetations, the infarctions and other parts. No other species of microorganism was found.

This micrococcus is very small, occurs mainly in pairs, sometimes in short chains, stains by Gram's method, grows in small, pale, grayish-white colonies on gelatine and agar, at first clouds bouillon, which then becomes clear with a whitish sediment, does not produce gas in glucose media, liquefies gelatine slowly and to some extent also blood serum, and is especially characterized by its behavior in milk, which it acidifies, coagulates and subsequently liquefies. It produces a milk-curdling ferment and also a proteolytic ferment, each of which is separable from the bacterial cells. It remains viable for months in old cultures and is tolerably resistant to the action of heat and anti-septics.

The micrococcus is pathogenic for mice and rabbits, causing either abscesses or general infections. Typical acute vegetative endocarditis was experimentally produced by intravenous inoculation of the organism in a rabbit and a dog, and the cocci were demonstrated in pure culture in the vegetations and other parts of these animals after death.

Although the micrococcus here described has some points of resemblance to the pneumococcus and *Streptococcus pyogenes* on the one hand and to the pyogenic staphylococci on the other, it is readily distinguished from each of these species by cultural features which have been described and which are so obvious that the differentiation of these species from our micrococcus need not be discussed in detail. We have searched through the records concerning microorganisms described in association with endocarditis and other diseases, as well as those isolated from water, soil and other sources, and have been unable to find a description of a micrococcus identical in all particulars with that here described. Such points as staining by Gram, liquefaction of gelatine, coagulation and peptonization of milk, served singly or in combination to distinguish our micrococcus from other forms which

in some respects might resemble it.\* We feel justified, therefore, in recognizing this organism as a new species and from its fermentative properties propose for it the name "Micrococcus zymogenes."

Micrococcus zymogenes must be added to the already considerable list of bacteria which have been found as the specific infective agents in endocarditis. That it was the cause of this affection in our case was conclusively demonstrated by its repeated isolation in pure culture from the blood during life, by its presence in pure culture and large numbers after death in the cardiac vegetations, the infarctions, and other parts of the body, and by the experimental proof of its pathogenic properties, and notably its capacity to produce vegetative endocarditis by intravenous inoculation in animals.

\* Dr. Norman MacL. Harris in this laboratory isolated in May, 1898, from material removed from an old cesspool in Baltimore, which had not been evacuated for twenty-two years, a micrococcus which in morphological and cultural characters is so similar to Micrococcus zymogenes that we at present believe the two organisms to be identical. The cesspool coccus at present liquefies gelatine and peptonizes milk somewhat more slowly than our coccus, but otherwise its cultural characters are indistinguishable from those of the latter, and morphologically the organisms are identical. Its pathogenic properties have not yet been tested.