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Dr. J. G. RUTHERFORD, Veterinary Director General.

BIOLOGICAL LABORATORY

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ACTINOBACILLOSIS

BY

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ACTINOBACILLOSIS

The possibility of this disease existing in Canada was first mentioned by the Veterinary Director General of the Department of Agriculture, Dr. J. G. Rutherford, in his annual report for 1902.[1] The confirmation of this suspicion was made by the writer in an official report dated June 23, 1903, and since that time three other identified cases have been studied and have also furnished the data upon which this Bulletin is based.

The non-identification of this disease in the past has been due to the fact that little or no original work has been conducted in Canada, on the infectious diseases of animals, and when such work has been accomplished the greatest difficulties have been encountered.

This disease, as the name implies, bears a similarity to the disease known as 'Actinomycosis' or 'Lumpy Jaw'; and in fact a study of its anatomical manifestations and pathological lesions would lead the uninitiated to believe they were dealing with that classic affection. Until 1900-01, the two diseases were considered identical, in fact no effort had been made to differentiate between them until it was shown by Lignières and Spitz[2] that two distinct diseases were being treated under the one name, 'Actinomycosis.' Their work was exhaustive and indicated that beside the streptothrix causing the classic Actinomycosis, a bacillus, having none of the characteristics of a dichotomous streptothrix was responsible for lesions exhibiting the same general character as are found in Actinomycosis, with the exception of their microchemical reactions, and, from its bacillary causative agent named it 'Actinobacillosis' (which means a ray-forming bacillus).

Nocard,[3] in 1902, identified this affection in France, showing also that its distribution was general in that country.

The disease studied by us is identical with that studied by Lignières and Spitz in the Argentine Republic, and M. Nocard in France, with the exception that the bacillus isolated from our cases has not in any instance shown the degree of virulence credited to that isolated by the investigators mentioned, hence we may safely assume that in the cases studied, we have been dealing with an attenuated form of the disease.

The history and extent of this affection in Canada has not been fully ascertained, but it is more than probable that a number of the cases known under the name of Actinomycosis, would, if investigated, be found to be due to the peculiar bacillus which we are about to describe.

It is not our purpose at this time to discuss the subject of Actinomycosis, as this disease has received consideration by various writers and we are not prepared to add any new scientific data to that already published on this affection. It is, however, our intention to present in a clear and concise manner the results of our investigations upon this newly described disease, 'Actinobacillosis,' that Canadian veterinarians and stock owners may know the essential characteristics of this disease which has been identified in Canada.

The history of the four cases identified by us is very brief and will be given in as full a manner as the data accompanying the material forwarded allows.

Case I.—An aged grade cow. Suffered considerably in fall of 1901 and spring of 1902. After being allowed to run to pasture for a time and delivered of her calf she seemed to make a good recovery. This year (1903) the symptoms returned in a more aggravated form and the animal was destroyed. The affected portion, consisting

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[1] Annual report of the Minister of Agriculture, 1902, p. 82.

[2] J. Lignières and G. Spitz, de L'Institut National Bactériologie Buenos Aires, Actinobacillose, Recueil de Médecin Vétérinaire, 1903.

[3] Nocard, Actinobacillose der zunge, Jhar. der Vet. Med. Berlin, LVI., Bd., p. 695, 1903 (Abstract).

of the pharynx, larynx and the upper part of the trachea, including the œsophagus and tumour mass (plate I.), was forwarded to the laboratory.

The material on arrival was looked upon as a tumour of non-infectious character and was immediately placed in a solution of formaldehyde until the pressure of routine work would allow its examination which was a few days later. On section the characteristic semi-fluid glue-like pus was revealed which is almost diagnostic. It was only at this time that the exact nature of the affection was suspected and this too late to obtain positive results from animal inoculation on account of the time the material had remained in the preserving fluid. Microscopic preparations of the pus revealed the peculiar clubs (plate III., fig. 1), and no portion of the smear preparation retained the colouring matter when treated by the method of Gram. Sections of the tumour cut in paraffine exhibited the same micro-chemical characteristics.

Case II.—This animal was a pure bred Shorthorn bull. He was examined by the local veterinarian in May. He had what was diagnosed as a small abscess in the left parotid gland. Potassium iodide was prescribed and continued for a long period, nevertheless, the abscess increased in size, the animal continued losing in flesh and breathed with some difficulty, due to the pressure on the larynx of the tumour mass. Later another abscess appeared on the hip near the tail. Owing to the condition of the animal and the progress the disease was making he was destroyed.

The material forwarded to the laboratory in this instance consisted of pus, taken at the time of opening the abscess in the region of the parotid gland. Laboratory animals (guinea-pigs and rabbits) were immediately inoculated. The first animal to succumb to the effects of the inoculation was a guinea-pig, death occurring on the nineteenth day after intra-peritoneal injection of the pus. At the autopsy this animal exhibited the characteristic lesions of the disease from which the bacillus was obtained in pure cultures.

Case III.—In this case the veterinarian was called to see the animal and owing to the extremely emaciated condition of the subject she was destroyed. It was noted that the tongue was not normal, in consequence of which it was removed and forwarded to the laboratory. (See plate II).

Arriving in a fresh condition animal inoculations were made subcutaneously, the first succumbing to the effects of the disease at the end of twenty-six days. The lesions at the autopsy were characteristic of the disease, and the bacillus was obtained from them in pure culture.

Case IV.—A growth appeared on the jaw of an animal which was being fattened for beef. This growth was supposed to have resulted from the kick of a horse. A portion of the mass was forwarded to the laboratory for examination.

On microscopical examination the lesions of Actinobacillosis were found, there being no filaments and no portion of the material retained the colouring matter when treated by the method of Gram.

Lesions.—The gross lesions seen in an infected animal are very similar to those of Actinomycosis, consisting principally of a fibrous tissue hyperplasia. In many instances the lesions can only be differentiated from those of Actinomycosis by their reaction to the various colouring matters used in preparing the material for microscopic examination, particularly to the method of Gram, decolourizing when treated with alcohol. The pus is characteristic, of a semi-solid consistency, glutinous, almost transparent and containing whitish granules which are scarcely visible to the naked eye. These granules when examined under the microscope exhibit 'bizarre' forms, which under high magnification show the peculiar bulb-like processes radiating from the mass. (Plate III., fig. 1.) Owing to the glutinous nature of the pus it is with difficulty drawn into the ordinary laboratory pipettes, which must be of large calibre. If successfully drawn into them, greater difficulty is experienced in removing it in the process of making cover-slip preparations, cultures or animal inoculations.

Cultural Characteristics.—Cultures are obtained direct from the pus with some difficulty, it being first necessary to crush the granular masses. It has been our practice to crush these particles against the side of the test-tube, using a heavy platinum wire for the purpose.

Morphology.—The bacillus causing Actinobacillosis resembles in a marked degree the bacillus of Fowl Cholera[4] studied by the writer in 1896. It is aerobic, facultative anaerobic, non-motile and in sizes varies, usually being from 1·0-1·8 μ long and 0·4-0·6 broad. A distinct polar arrangement of the protoplasm is noted in the hanging drop preparation. It stains with the ordinary aniline dyes, particularly those which are acidulated ; but does not retain the colouring matter when treated by the method of Gram.

Broth.—Culture in beef broth[5] show a slight colouring of the medium in twenty-four hours at 37°C. This cloudiness increases and after some days a slight sediment is noted. No scum has appeared on the surface of the medium. The addition of glycerine, up to five per cent, does not influence the growth.

Gelatin.—Only occasionally has it been possible to obtain any growth in gelatin, and even this has been very slight, appearing as very fine points, visible only on magnification. These points may appear deep in the medium, along the line of stab or near the surface. No liquifaction of this medium has been observed.

Agar.—Upon agar, small translucent colonies of one millimetre in diameter are noted at the end of twenty-four hours. The edges of these colonies are granular. In stab cultures growth is observed in the depth of the medium as well as on the surface. Colonies deep in the medium along the line of stab are finely granular and do not extend into the surrounding medium.

Gas Production.—No formation of gas has been noted in saccharose, glucose or lactose broth. There is a clouding of these media.

Milk.—Milk to which litmus and lactose have been added exhibits no change in reaction, nor is there a coagulation of the medium. This medium furnishes conditions favourable to the development of this organism.

Serum.—In liquid serum a floculent growth is observed in from twenty-four to forty-eight hours, which falls to the bottom of the tube. No general turbidity of this medium is observed.

Potato.—Upon alkaline potato a slight growth is noted after four days, appearing as small white colonies on the medium. Potatoes which are acid present no growth even after prolonged incubation.

Egg media.[6]—It was with egg media that the greater portion of our investigations with this disease were carried out, and it was the only medium used in the isolation of the bacillus. In the process of isolating the germ from the affected tissue

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[4] C. H. Higgins, Notes upon an Epidemic of Fowl Cholera, Jour. Expt. Med. Vol. III, No. 6, 1898.

[5] Preparation of broth:—

Leibig's extract of beef....	5·
Sodium chloride..	10·
Witte's peptone..	5·
Distilled water..	1000·

This is boiled one hour, neutralized with potassium hydrate using phenolpthalein as an indicator and again boiled for thirty minutes and filtered. In neutralizing the medium is left slightly alkaline to compensate for the change which takes place in sterilizing in the autoclave. This broth was the basis of gelatine and agar media.

[6] M. Dorset. The use of Eggs as a Medium for the Cultivation of the Bacillus Tuberculosis. Annual report of the Bureau of Animal Industry, United States, 1901, p. 574.

of an experimental animal, the precautions noted by Theobald Smith for the isolation of the tubercle bacillus were observed. A portion of the tissue taken under such precautions was placed in the tube, partially crushed with a heavy platinum wire and smeared over the surface of the medium. A growth may appear in three days, but was in some instances not observed previous to eighteen days' incubation at 37°C. The first colonies appear as very small white dots raised from the surrounding medium, and in growing, form a mass which has the appearance of half a sphere, never attaining a diameter greater than two millimetres after prolonged incubation. If these first colonies are touched with the platinum wire, they are found to be rather hard and firmly adherent to the underlying medium, but if a smear preparation is made, the characteristic bacilli are found. If streaks on the medium are made from these first colonies, either in the same tube or transfers, observation after twenty-four hours reveals many small colonies, none exceeding one millimetre in diameter. After prolonged incubation the colonies grow very little larger, but become more numerous, gradually filling the intervening space, and finally form a white streak on the medium.

Indol.—A trace of indol is present in old broth cultures.

Agglutination.—Serum of experimental animals affected with Actinobacillosis causes a clumping of the bacilli in the hanging drop preparation, while that of other animals not infected causes no agglutination reaction.

Vitality.—The bacillus is destroyed in ten minutes at 62°C. Growths are obtained only at incubator temperature (37°C.), but may occasionally be obtained in a very slight degree at room temperature (20°C.). Tissue kept frozen for seven days, during which time the temperature ranged from —0° F. to —20° F., proved fatal to guinea-pigs on subcutaneous inoculation, in 25, 28 and 45 days respectively.

ANIMAL INOCULATIONS.

Guinea-pigs.—Guinea-pigs inoculated intra-peritoneally with pure cultures or pus die in from nineteen to thirty-one days of a generalized actinobacillosis. We have been unable to produce death in a shorter period with a general peritonitis, which fact indicates, as already stated, that we are dealing with an attenuated virus.

The lesions presented at the autopsy are characteristic and very interesting, being entirely different from those observed, the result of other infective agents. Small pearly-white nodules appear just beneath the peritoneal and pleural membranes, varying from 1·0–5·0 mm. in diameter. (Plate V.) The liver presents lesions throughout its substance, the surface being mottled. The spleen shows, usually, a varying number of nodules. The great mesenteric fold of the omentum has in every instance been the seat of extensive lesions, and, in some cases has a thickness of one and one-half centimetres and a length of eight centimetres. The kidneys present nodules beneath their serous covering, but none have been observed in the substance of the organ. The diaphragm may contain numerous nodules beneath its serous surfaces. The stomach and intestines usually present nodules on their serous surfaces, varying from 1·0 mm. to 0·5 c.m in diameter. Ulcers are usually present on the mucous surface of the stomach varying in size from 3·0 to 5·0 mm. in diameter. Ulcers were also noted in the intestine, particularly in the cæcum and large intestine. The lungs present greater or less involvement of their structure; in some instances there being a few superficial nodules, while in others the lesions are general throughout the tissue of these organs. Serous fluid has been present in both the thoracic and abdominal cavities, but is not constantly found in either. Nodules have been observed on the surface of the heart and in the pericardial membrane. An excessive amount of fluid may or may not be present in the pericardial sac. No lesions of the endocardium have been observed. At the point where the needle enters the peritoneal cavity there is always an extensive nodular manifestation in the abdominal wall beneath the peritoneum. This is well shown in plate V. The various lymph glands are usually enlarged, and present lesions.

Subcutaneous inoculation is usually followed by the same general lesions above mentioned. There is usually an abscess formed at the point of inoculation, and the lymph glands in the immediate neighbourhood are greatly enlarged. There may be no generalized infection where this method of inoculation is practised, death being due to toxic poisoning. This method of inoculation requires a somewhat longer period to result fatally, usually being from twenty-five to thirty-eight days.

Rabbits.—Rabbits, inoculated intra-peritoneally, present lesions very similar to those seen in guinea-pigs. There is a generalized actinobacillosis, in which the thoracic and abdominal viscera are involved to a greater or less extent. (Plate VI.) There is usually ulceration of the intestinal tract, more particularly of the cæcum and large intestine. In one instance this was very extensive. (Plate VII.) The serous membranes of the thoracic and abdominal cavity are extensively involved. The lesions in the diaphragm and mesentery are particularly well shown in plate VIII. It may be also observed in this same plate that the pericardial membrane contains nodules, the distinctness of which was impaired in photographing and subsequent reproduction. Inoculated intra-peritoneally with either pure cultures or pus, rabbits die in from fifty-one to seventy days. In one instance one hundred cubic centimetres of fluid was contained in the abdominal cavity, and in this fluid the characteristic tuft formation was demonstrated on microscopic examination.

We have not studied the virulence of this germ for other animals at this laboratory.

Microscopic examination.—The microscopic examination of the pus and tissues from animals affected with Actinobacillosis requires special technique to differentiate their various characteristics. The best results are obtained by the use of eosin and methylene blue, and the method described by Lignières and Spitz[7] has given excellent results with both the smear preparations from pus and paraffine sections of affected tissue. The methods of Gram and Wiegert also give good microscopic preparations. A saturated solution of eosin may be used, followed by Unna's alkaline methylene blue. Good microscopic preparations of the pus may be obtained by the use of Romanowsky's stain as modified by Dutton and Todd.[8]

In fresh pus the tufts are not easily distinguished, but when squeezed between the slide and coverglass are clearly visible, even to the naked eye. They are of a whitish-gray colour, and may be more easily examined if a little picro-carmine glycerine is placed at the edge of the coverslip, as the tufts stain yellow with picric acid and the rest of the field will assume a reddish tinge.

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[7] Lignières and Spitz. Actinobacillose, Recueil de Médecin Vétérinaire, September 30th, 1902. Their method is as follows:

Eosin, watery solution (Hoechst).. 1 part
Borrell's Blue.. .. .. 1 part
Water.. .. .. 8 parts

Mix just before use and filter rapidly. Suspend sections attached to slides or coverslip preparations, upside down over the staining dish and allow the stain to saturate them from below, thus avoiding the precipitate which is formed in the staining material. Stain in this solution for thirty minutes. Wash thoroughly in water. Use 10 per cent solution of tannic acid, which will cause colour of the section to brighten. Wash again in water. Dehydrate in alcohol. Clear with oil of caryophyllae and mount in xylol balsam. The stain, as above prepared, spoils within an hour and almost completely loses its staining qualities.

[8] Dutton and Todd. Trypanosomiasis Expedition to Senegambia, Liverpool School of Tropical Medicine, 1902, p. 3. Modification of Romanowsky's stain.

Solution A. Medicinal methylene blue (Hoechst).. .. .. ·5 Grammes
Saturated solution of chemically pure borax.. .. .. 0·5 c. c.
Incubate four days at 37° C., then add absolute alcohol.... 50· c. c.

Solution B. Eosin, Extra B.A. Crystals (Hoechst).. .. .. 25·0 Grammes
Distilled water.. .. .. 50· c. c.
Absolute alcohol.. .. .. 50·0 c. c.

For use dilute with water, one part of stain to nineteen parts of water. Mix equal parts of diluted stain in a flask and pour immediately into a staining dish. Stain three to six minutes. Wash quickly but thoroughly in tap water and dry in the air without the aid of heat.

Lesions in the various organs and tissues exhibit the same general characters as are exhibited by the tufts in the pus. The peculiar bulb-like processes are seen to extend toward the surrounding tissue, similarly as is the case in actinomycosis. Immediately surrounding the mass of the lesion is an inflammatory area, its extent depending upon the nature of the lesion. (Plate IV., figures 1 and 2.)

Infectiousness.—Actinobacillosis is an infectious disease, capable of communication by direct inoculation. We are not prepared at the present time to indicate the degree of danger through co-habitation, but from the nature of the infective agent we believe that this danger is perhaps slightly greater than is the case with actinomycosis. We have not found in any of the material indication of grains.

Treatment.—In this bulletin on the subject of Actinobacillosis it is fitting that something be said concerning the treatment of affected animals. We have conducted no experiments with this end in view, although we have such under consideration, but we have the results of other workers, who indicate treatment similar to that which is pursued in cases of actinomycosis, consisting principally in the administration of large doses of potassium iodide. This treatment, while beneficial, will have no ultimate results unless prescribed early in the manifestation of the affection.

From the fact that in the majority of cases the lesions are located in the region of the larynx, and from the extensive tumour formation respiration is seriously interfered with, it is easily understood why the treatment must be commenced early. If the disease process has extended too far, the condition of the animal is such as to make treatment an unprofitable investment, for we have beside the actual lesions, the toxine poisoning to deal with.

EXPLANATION OF PLATES.

Plate I.—Tumour formation from case I., showing the distorted œsophagus, &c.,

Plate II.—Case III. Actinobacillosis of the tongue.

Plate III.—Figure 1.—A tuft from fresh pus of case I. Coverslip preparation stained by the method of Lignières and Spitz. Highly magnified (x 1,500 diameters). Figure 2.—Bacilli of Actinobacillosis. Coverslip preparation from a culture on whole egg media. Stained with carbol-fuchsin. Highly magnified (x 1,000 diameters).

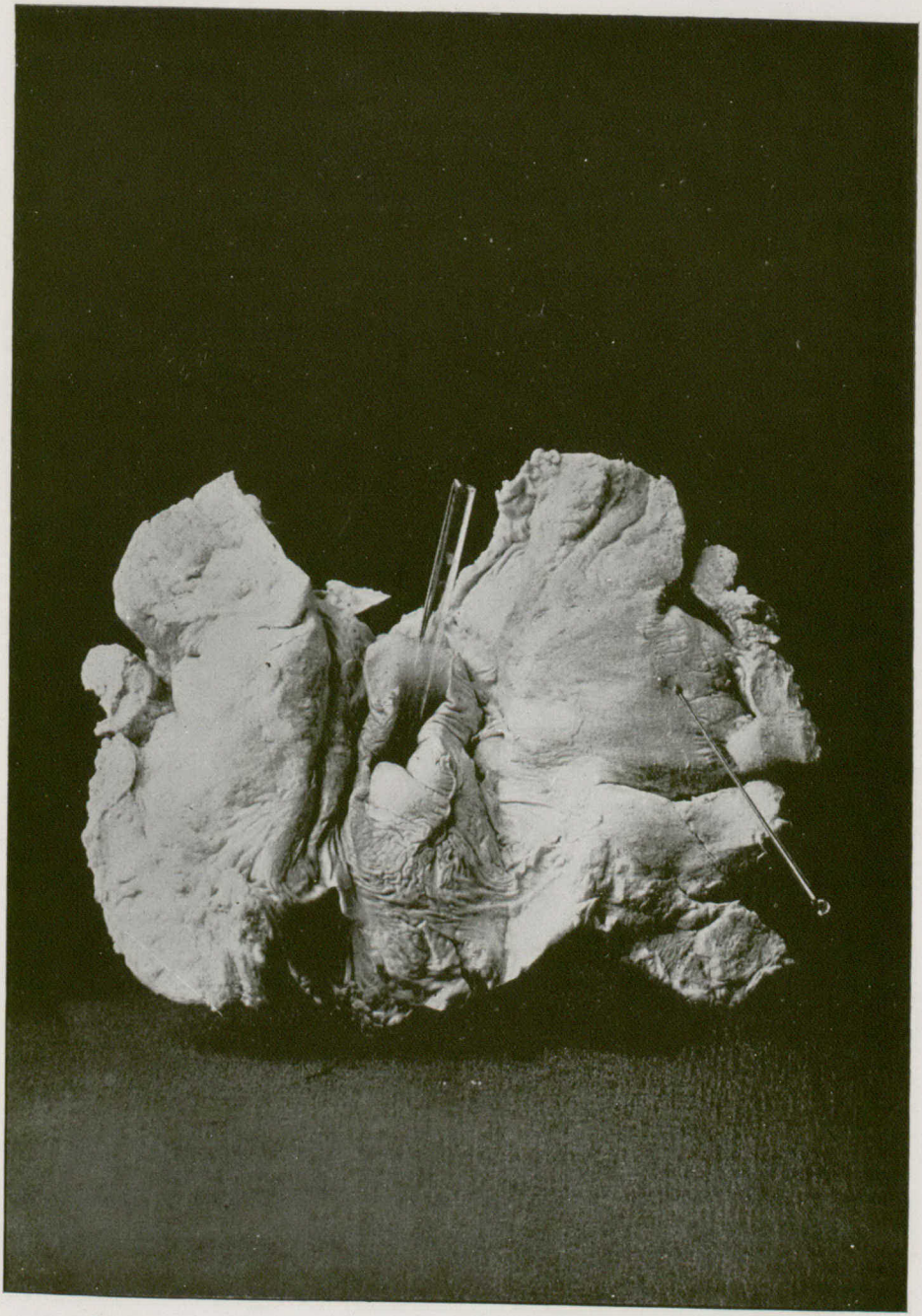
Plate IV.—Figure 1.—Section from the tumour mass of case I., cut in paraffine, and stained by the method of Lignières and Spitz. Highly magnified (x 1,000 diameters). Figure 2.—Section of tumour from case IV. Section cut in gum on a freezing microtome. Stained by the method of Gram, but not fully discoloured. Highly magnified (x 1,000 diameters).

Plate V.—Guinea-pig infected with Actinobacillosis material from case II. Inoculated intra-peritoneally with a pure culture. Death in twenty-two days. Note lesions on peritoneal surface, also in lung, liver, spleen, and great mesenteric fold, which is greatly enlarged. Also note nodules on peritoneal surface of intestines, &c. This is characteristic of the condition at autopsy in guinea-pigs inoculated either with pure cultures or with pus from an affected animal.

Plate VI.—Rabbit showing lesions of Actinobacillosis. Inoculated with a pure culture obtained from case II.

Plate VII.—Intestines of a rabbit affected with Actinobacillosis. Inoculated with cultures obtained from case II. The animal from which the intestines were taken died in sixty-eight days of a generalized Actinobacillosis.

Plate VIII.—The diaphragm, with the pericardial sac attached, and two portions of the mesentery showing lesions of Actinobacillosis. The large portion is of the mesentery, with spleen attached. The small portion is also of the mesentery. The diaphragm is easily distinguished from its shape. This material was taken from a rabbit, and is two-thirds actual size.



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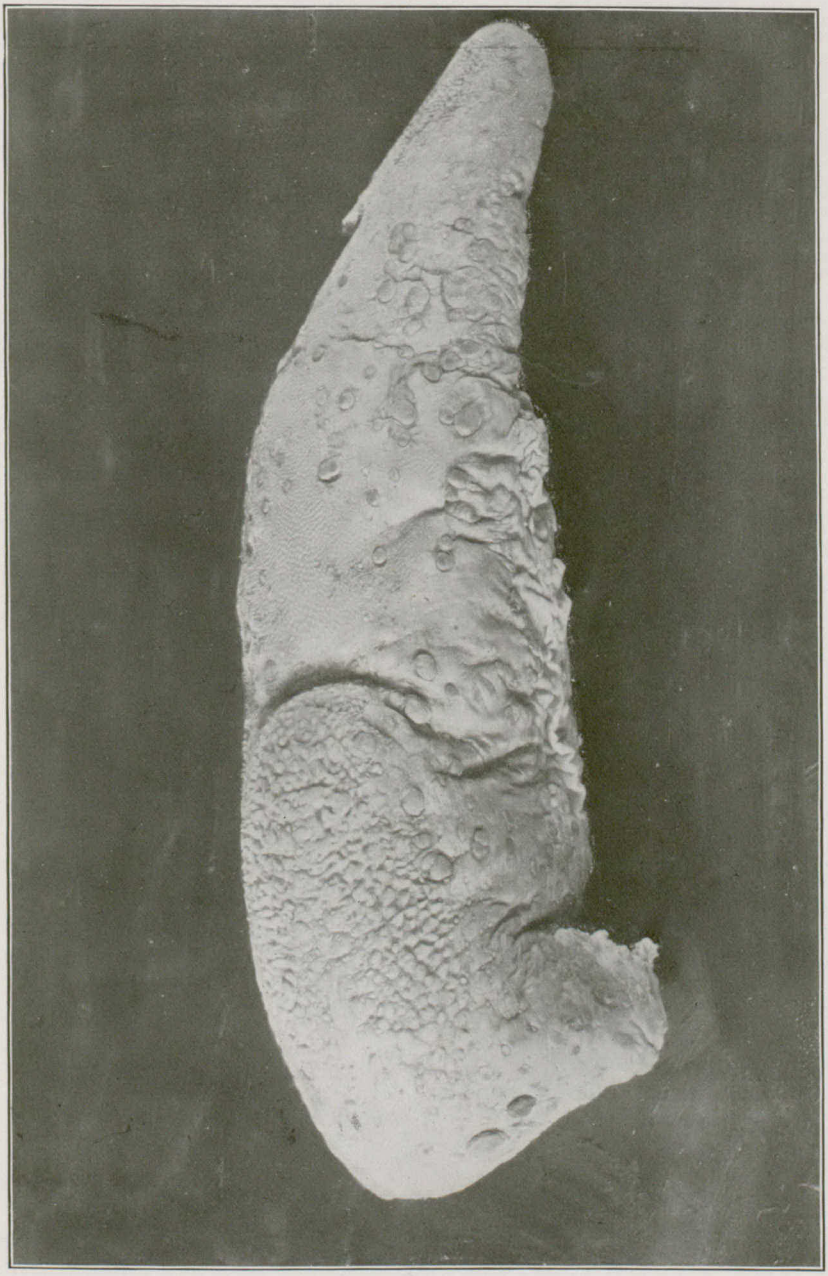

FIG. 1.

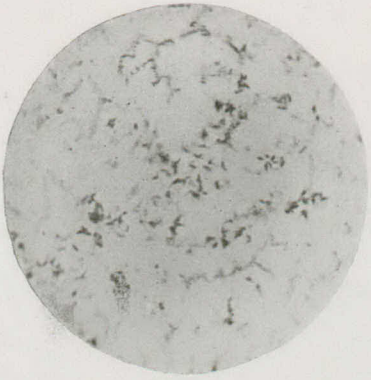

FIG. 2.

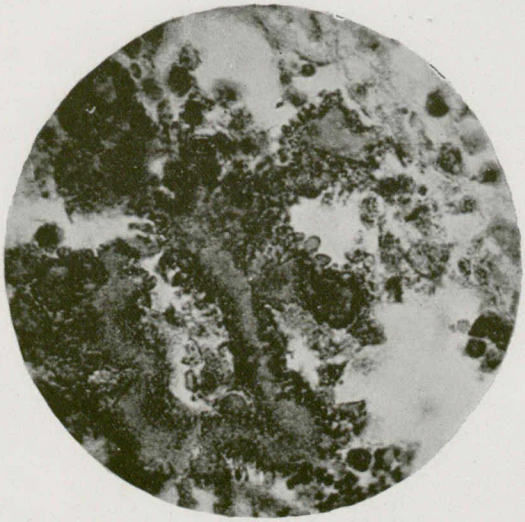

FIG. 1.

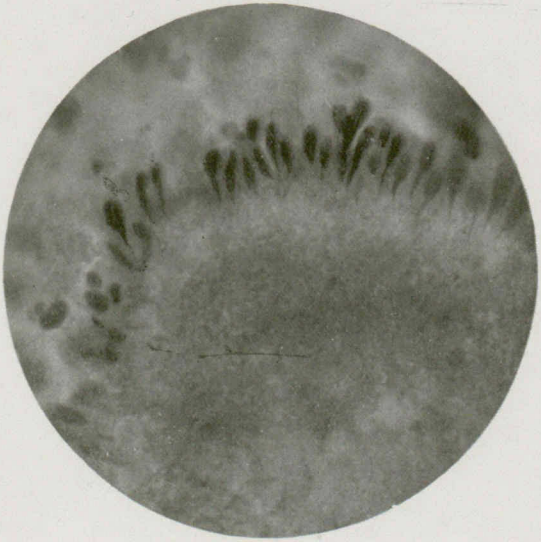

FIG. 2.