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GLANDERS: ITS DIAGNOSIS AND PREVENTION

TOGETHER WITH A
REPORT ON TWO CASES OF HUMAN GLANDERS OCCURRING IN MANILA AND SOME NOTES ON THE BACTERIOLOGY AND PLEOMORPHISM OF BACTERIUM MALLEI

BY

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LETTER OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, November 10, 1904.

SIR: I have the honor to transmit herewith and recommend for publication an article entitled "Glanders: Its Diagnosis and Prevention, together with a Report on Two Cases of Human Glanders Occurring in Manila and some Notes on the Bacteriology and Pleomorphism of Bacterium Mallei," by Wm. B. Wherry, M. D., Bacteriologist, Biological Laboratory.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory,
Acting Superintendent Government Laboratories.

Hon. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

GLANDERS: ITS DIAGNOSIS AND PREVENTION.

TOGETHER WITH A REPORT ON TWO CASES OF HUMAN GLANDERS OCCURRING IN MANILA AND SOME NOTES ON THE BACTERIOLOGY AND PLEOMORPHISM OF BACTERIUM MALLEI.

By Wm. B. Wherry, M. D., *Bacteriologist, Biological Laboratory.*

INTRODUCTION.

General nature and distribution of the disease.[1]—Glanders is a specific, contagious disease occurring naturally in horses, mules, and asses and sometimes transmitted to man and other animals. It may run an acute or chronic course which is characterized clinically by more or less grave constitutional disturbances, and pathologically by the formation of small nodular areas of leucocytic infiltration, especially in the mucous membranes of the upper air passages, the lungs, and the skin.

The specific organism which causes the disease was discovered in 1882 by Löffler and Schütz, who named it *Bacterium mallei* and established its etiologic relationship by reproducing the disease in animals with pure cultures. Since then an immense amount of work carried on in various parts of the world has resulted in valuable information concerning its diagnosis and control.

The disease has a very wide geographical distribution, existing throughout the greater part of the civilized world. Insular places, if far from the mainland and free from the importation of horses, usually escape. Thus glanders is very rare in Iceland and in the

[1] In the preparation of this brief review of the characteristics of the disease I have excerpted to a greater or less extent from the articles cited at the end of this bulletin. For fuller details one should consult the works of Councilman and Moore, where, also, further bibliographical references will be found.

Faroe Islands. In Australia, Tasmania, and New Zealand it is reported to be unknown (Moore.)

Prevalence in Manila.—The following statistics were obtained through the kindness of Maj. E. C. Carter, Commissioner of Public Health, from the report of Dr. John G. Slee, chief of the veterinary division of the Board of Health for the Philippine Islands:

During the year between September 1, 1903, and August 31, 1904, 3,764 horses were imported into Manila. Of these 165 were condemned and cremated for glanders and 42 condemed and cremated for surra. All suspicious cases were tested with mallein.

Again, through the kindness of Dr. John Ernst, veterinarian, Quartermaster's Department, United States Army, it is possible to state the number of public animals that died of or were destroyed for glanders and surra in the Philippine Division during the fiscal year ending June 30, 1904, as follows:

Animal.	Number.	Died of or killed for glanders.	Killed for surra.
Horses	4,759	294	132
Mules	2,183	75	70
Total		369	202

It would be a mistake to assume from these statistics that glanders was more prevalent than surra during the past year, however that may be. The first series deals with imported horses only, and the second series only seems to indicate the greater ease with which surra can be controlled when truly military quarantine regulations are put into operation.

Susceptibility of animals.—Among the *Equidæ,* asses are most susceptible to the disease, mules almost as much so, while horses are slightly more resistant. Man seems to be almost as susceptible as the horse. Natural infection occurs only among the *Equidæ* and in man. The disease may be transmitted by means of inoculation to quite a number of other animals—goats, sheep, young dogs, cats, guinea pigs, rabbits, and field mice. A number of instances of the infection of wild animals in menageries have been reported, especially among the carnivora fed on the meat of glandered horses. Swine and pigeons are very slightly susceptible. Cattle, white mice, rats, and domestic fowls seem to be immune.

Modes of infection.—The specific cause of the disease has never

been found outside the animal body, and the result of many investigations show that it has feeble powers of resistance under such conditions. It occurs in large numbers in the discharge from the nostrils, in the pus from the specific ulcers, in the more recent nodules in the internal organs, and occasionally in the blood in acute cases of glanders. All evidence points to its direct or indirect transmission from animal to animal, by contact, by inhalation, by food and drink, and by infected harness, etc., through skin abrasions.

In man it occurs most frequently in those who have much to do with horses—veterinarians, stable boys, etc. It may be transmitted from man to man. Washerwomen have been known to receive infection from the clothes of a glandered patient. Infection has occurred from the material thrown off by the snorting of a glandered horse.

Types of the disease in horse and man.—The disease is termed *acute or chronic glanders* when it chiefly involves the mucous membrane of the nares, the adjoining lymphatic glands, and the lungs; and *acute* or *chronic farcy* when the process is limited to the skin and its lymphatics.

Acute glanders is common in the ass and mule, but infrequent in the horse. It is the common type of the disease in man and is invariably fatal; it rarely, if ever, passes into the chronic form. The onset and the course of the disease are very much alike in the horse and man. The period of incubation is usually from three to five days, when inflammatory symptoms appear at the site of infection, accompanied by symptoms of general febrile disturbance. A day or so later the mucous membrane of one or both nares is involved, becoming intensely hyperemic and often showing small punctiform hemorrhages. A copious discharge flows from the nostrils. At first this is thin and watery, but later becomes mucopurulent and may be streaked with blood. Small pustular nodules appear on the inflamed mucous membrane. These may be discrete and, upon opening, leave small, shallow, reddish areas of ulceration, which rapidly enlarge and may become confluent, forming large, irregular, ulcerated surfaces covered with muco-pus. Further, *in the horse,* the submaxillary lymphatic glands become enlarged on the affected side, nodules may form in the lungs, and after a rapid course death occurs, usually from suffocation, in one or two weeks. *In man* the mucous membrane of the nose is often involved at an early stage and this process is followed or accom-

panied by intense pains in the joints and muscles. Abscesses form, most commonly in the biceps, the flexors of the forearm, the rectus and the pectoral muscles. Sometimes very early in the course of the disease, or again, just before death, an eruption of papules, which soon become pustular, appears on the skin, especially on the face and about the joints. This has been mistaken for variola and the patients isolated and treated as smallpox cases. The eruption differs from that of smallpox, however, in the great variation in size and the irregular distribution of the pustules, which, further, are not umbilicated.[1] The ulceration of the nasal mucosa may end in necrosis of the deeper structures, accompanied by great swelling of the nose and face. It is invariably fatal in eight or ten days. Subacute cases have survived as long as thirty days.

Chronic glanders.—In chronic glanders the onset is often insidious and the course of such an indefinite type that it may be impossible to diagnose the disease without the use of special tests, which will be described under the subject of diagnosis.

The first symptom noted in the horse may be a sticky mucopurulent secretion from one or both nostrils, with the formation of small, grayish nodules on the upper portion of the nasal septum. These vary in size from that of a pin head to that of a pea, and are often surrounded by a zone of congestion. They soon break down and give rise to discharging ulcerated areas (see Pls. I and II), which may remain superficial or may deepen and even end in perforation of the underlying cartilage and bone. When these deeper ulcers heal they leave irregular, star-shaped cicatrices, which are considered of diagnostic import from a clinical point of view. (See Pl. VII.)

Such ulceration may be found in the frontal sinuses, eustachian tubes, larynx, etc. The submaxillary glands are often involved and in some cases may be the only apparent seat of infection. Along with the nasal and glandular involvement, the lungs are often affected. Here nodular or diffusely infiltrated lesions may form, accompanied, clinically, by coughing.

If a lung from such a case of glanders be examined post mortem and the hand passed over the pleural surface, few or many

[1] In one of our cases, Case II (see Pl. IV), several of the pustules showed depressed centers, but the umbilication was not so distinct as that seen in smallpox.

nodules may be felt. When numerous, as described by McFadyean, the lung feels like a bag full of peas. The nodules vary in size from 1 to 5 or 6 millimeters in diameter and on section appear as rounded, grayish areas surrounded by a zone of congestion and often by areas of lobular pneumonia. They always undergo caseation or encapsulation. Sometimes they become calcified.

The diffusely infiltrated lesions in the lungs may form sarcoma-like tumors, which may become so indurated as to form fibroma-like tumors, or they may break down and become gangrenous. In the more chronic cases the lesions may be confined to the lungs alone, or to a testicle or to some internal organ, and the only symptoms present may be a cough or an orchitis, or just a "general loss of condition." These are the cases in which special methods of diagnosis must be employed.

Chronic glanders is rare in man and is difficult to diagnose. It has been mistaken for chronic coryza. The most common form is due to local infection of an extremity, followed by lymphangitis and subcutaneous and muscular abscesses. In some cases a chronic ulcer or a muscular abscess may be the only evidence of the disease. It may last for months or years and may end in recovery or in the acute form of the disease.

In *farcy* the inoculation of the virus usually takes place through some abrasion of the skin. Nodules are then formed in the papillary layers of the dermis, in the cutis, and in the subcutaneous and intermuscular tissues. The nodules may turn into abscesses and discharge externally. The neighboring lymphatic vessels are inflamed and swollen, and along their course secondary subcutaneous enlargements are formed—the so-called farcy buds. The neighboring lymphatic glands are involved, and rarely secondary chronic farcy develops. Embolic foci form in the spleen, and more rarely in the liver, testicles, kidneys, brain, muscles, heart, and bones.

In acute farcy in man pains and swelling in the joints and muscular abscesses are common. Nasal ulcerations and eruptions on the skin do not usually occur. The acute form is fatal in a large proportion of the cases in from twelve to fifteen days. In the chronic form localized tumors, which undergo abscess formation and ulceration, may be the only symptoms of the disease and may last for months or years and end in recovery or, occasionally, in acute glanders.

THE DIAGNOSIS OF GLANDERS.

This is obviously of great importance to those interested in the prevention of its dissemination among horses and men. The acute cases present but little difficulty, as the symptoms are sufficiently pronounced to enable one to arrive at a diagnosis. The subacute and chronic cases must be differentiated from a variety of chronic nasal and lymphatic disorders in the horse, such as chronic coryza, blastomycetic pseudo-farcy,[1] etc.

In man glanders should be thought of in chronic ulcerations of the extremities, chronic coryza, muscular abscesses, and various joint involvements, especially if the occupation of the patient throws him into contact with horses.

In *acute glanders* the diagnosis may be confirmed by microscopical examination of the secretions of the nose or the farcy buds in the horse and of similar secretions in human cases. Whether glanders-like bacteria are found or not, guinea pigs should be inoculated according to the method of Strauss as described below.

In subacute or chronic cases two reliable methods are in vogue, (1) animal inoculation and (2) the injection of Mallein.

(1) *Animal inoculation.*—In 1886 Strauss showed that when material containing the *Bacterium mallei* is injected into the male guinea pig the animal develops after a time an orchitis, with the formation of nodules in the scrotal peritoneum, which varies in severity according to the quantity or virulence of the bacteria injected and the manner of inoculation. The nasal secretion from a suspected animal or scrapings from the nasal ulcers, the farcy buds, the chronic ulcers, etc., should be examined microscopically. Glander-like bacteria may or may not be seen. If comparatively few bacteria are present a small amount of the secretion is emulsified with bouillon or salt solution and injected into the abdominal cavity of a male guinea pig. If *Bacterium mallei* is present one or both testicles of the animal become inflamed and immovable and may reach the size of a walnut in three or four days. When a distinct orchitis has developed the animal is chloroformed and further examinations made microscopically and culturally.

[1] See Bulletin No. 1, Biological Laboratory, Bureau of Government Laboratories, by R. P. Strong: Preliminary Report of the Appearance in the Philippine Islands of a Disease Clinically Resembling Glanders.

When the inoculated material contains large numbers of other bacteria, as is often the case, intraperitoneal inoculation may result in death from peritonitis. In such cases it is best to inoculate two guinea pigs, one intraperitoneally and the other subcutaneously. In subcutaneous inoculation local ulceration is usually followed in a few days by involvement of the testicles or other organs.

Nocard has shown that this test is not altogether infallible, as a bacillus is found in another disease of horses, characterized by ulceration of the extremities, that produces lesions, closely resembling those produced by *Bacterium mallei,* in the testicles of guinea pigs.

Frothingham, in reviewing his extensive experience with the method of Strauss, had only four absolute failures out of a large number of tests. His statistics show that, where in a few instances no scrotal lesions are produced in the guinea pig, glanders abscesses may occur in other parts of the body. In discussing the fallacies of the test he mentions the bacillus found by Kutscher in the nasal secretion of a glandered horse which closely resembles *Bacterium mallei* in its morphology and produces similar lesions in the testicles of guinea pigs. However, the former organism yields a white growth on potato, which is easily distinguished from the amber-colored, honey-like growth of the bacterium of glanders. The *Bacillus pyocyaneus* may also produce a somewhat similar growth on potato, but is easily distinguished by its motility in the hanging drop. He emphasizes the fact that in the use of the Strauss method a positive result means everything, while a negative one means nothing.

(2) *By the injection of mallein.*[1]—Mallein is prepared, after the manner of tuberculin, by growing a freshly isolated culture of *Bacterium mallei* in glycerine bouillon for from four to six weeks. The culture is then sterilized in the autoclave on three successive days and filtered through a Pasteur filter.

When an animal affected with glanders is inoculated (in the horse, usually in the subcutaneous tissue of the neck) with a sufficient amount of mallein, the size of the dose varying according to whether the mallein is concentrated or not (0.5 to 5 cubic

[1]This substance is prepared in Manila at the Government Serum Laboratory and can be obtained by applying to the Bureau of Government Laboratories.

centimeters), a specific and definite local and general reaction is produced.

According to Moore the reaction is as follows:

In a few hours there forms at the place of injection a hot, inflammatory swelling. It is painful and in the case of glanders quite large.[1] * * *

From all sides of the swelling there may radiate wavy lines consisting of swollen lymphatics, hot and painful when touched, extending toward the adjoining glands. When the mallein injection is made aseptically this swelling never suppurates, but it increases in size during the period of from twenty-four to thirty-six hours and persists for several days, when it gradually diminishes and finally disappears at the end of eight to ten days. With the appearance of the local swelling the patient becomes dull and dejected, the eyes have an anxious expression, the coat is lusterless, the flanks contracted, the respiration hurried, the appetite is impaired, frequent shudders are observed to pass through the muscles of the legs, and sometimes the trunk is subject to violent convulsive movements. The most active and fractious horses become listless and indifferent to their surroundings. These general phenomena constitute what the French call the organic reaction, but they are not always so clearly marked. Difference in their intensity is observed, but they are never completely absent. * * *

The temperature reaction never fails to show itself. About eight hours after the injection the temperature of a glandered horse usually rises 0.5°, 2°, or even 2.5°, and even more above the normal. The rise in temperature usually attains its maximum between the tenth and twelfth hour, occasionally not until the fifteenth, and more rarely not until the eighteenth hour. An important fact to note is that the reaction called forth in glandered horses by the injection of mallein persists for from twenty-four to forty-eight hours, and in some cases the temperature remains above the normal for even a longer time. In practice it is advisable to take the temperature of the suspected animal two or three times before the injection of the mallein, and every two hours beginning at the eighth and going to the twentieth hour after the injection. It is often sufficient for diagnostic purposes to take the temperature but four times, viz, at nine, twelve, fifteen, and eighteen hours after the injection. * * *

In healthy horses the injection of mallein, even in a much larger dose, produces no effect on the temperature or the general condition of the animal. * * *

There is produced, however, at the point of injection, a small, edematous swelling, somewhat hot and painful to the touch, but the edema instead of increasing diminishes rapidly and disappears in less than twenty-four hours.

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[1] McFadyean deems this local reaction of the greatest diagnostic value. In the case of healthy horses it is rarely more than 3 inches across, reaches its maximum in fourteen to sixteen hours, and usually disappears within twenty-four hours. In glandered horses, on the other hand, the swelling is rarely less than 5 inches across, often twice this, and continues to increase for twenty-four hours.

When the local reaction is carefully watched along with the temperature there is little danger of mistaking a rise of temperature due to other causes as a part of the specific reaction. The test should never be made on animals already exhibiting an abnormal temperature.

THE PREVENTION OF GLANDERS.

There is probably less excuse for the existence of glanders among our domestic animals than of any other disease with the possible exception of surra. The specific cause of the malady has such low powers of resistance outside the animal body that the eradication of existing foci should be a comparatively easy task.

The killing and cremation of the bodies of all acute cases occurring in horses or mules is naturally the wisest course, since they invariably result fatally. With regard to the proper disposal of subacute and chronic cases diagnosed by means of mallein, there seems to be a considerable difference of opinion. Nocard, the great French authority, said:

> We ought, therefore, to confine ourselves to the destruction of those which, in addition to the reaction, present some clinical indication of the disease, such as ulceration of the nose, indurated glands, suppurative lymphangitis, varicocele, or other pronounced manifestations of the disease. The animals not showing physical signs of affection must simply be removed from among the healthy horses and subjected from time to time—say, every two months—to the mallein test. If any of these eventually show clinical signs of glanders they ought to be slaughtered at once. On the other hand, those animals which have stood two successive doses of mallein without reacting ought to be considered definitely cured, restored to their places, and placed at the free disposal of their owners.

It is generally agreed that if a single horse in a stable reacts to mallein all should be subjected to the test, the infected animals isolated, and the yards, stables, harness, and whatever else may have come in contact with the horses disinfected.

The most efficient, the cheapest, and, if proper precautions are taken, a perfectly safe method of disinfecting stables and yards is by fire. Stiles (1902) reported very favorably upon the disinfection of kennels, pens, and yards by fire generated in the so-called "cyclone burner," as described by Forbush and Fernald (1896). Briefly the apparatus consists of a portable tank and pump from which paraffin-gas oil is driven through a hose (such as is used for the delivery of oil), to which is attached a pole, consisting of

an iron pipe 12 feet long, which is protected by a covering of wood, and to the end of which is attached a cyclone nozzle. The fine spray from the nozzle is ignited and the resulting fierce flame passed over the surfaces to be disinfected. The thorough wetting, with water, of all such surfaces would practically abolish the danger from fire and at the same time increase the germicidal action of the heat.[1]

All harness and similar material which has been used by or come in contact with the glandered horses should be thoroughly disinfected. This may be accomplished by first removing all foreign matter, such as dirt, dressing, etc., and then by scrubbing with soap and hot water, followed by a thorough scrubbing with a milky emulsion of creolin or a solution of 4 per cent carbolic acid.

TWO CASES OF HUMAN GLANDERS OCCURRING IN MANILA.

Case I.

History (furnished by Maj. J. M. Bannister of the First Reserve Hospital, by permission of the chief surgeon).—Henry Marshall, an American negro, 27 years old, who had been a teamster for the Manila Street Car Company, was admitted to the First Reserve Hospital on May 29, 1904, with a diagnosis of articular rheumatism. When about 20 years old he had suffered from an attack of articular rheumatism, involving the elbows and the knees, which terminated in about three months in recovery. There is no history of further illness until the present attack, which began with daily severe chills. At this time he entered the San Juan de Dios Hospital, where he remained for two weeks, suffering with high fever and rheumatic pains in the knees, elbows, and ankles. He was next removed to the First Reserve Hospital, on admission to which he was weak, was suffering much pain, had a temperature of 105.2° F. and perspired profusely.

While at the First Reserve Hospital he had a remittent temperature varying between 101° and 105° F., with an irregular morn-

[1] This method appeals to one as being not merely cheap and easily applied but as one which might be adopted for the disinfection of native quarters and nipa shacks and the ground around and beneath them during the prevalence of various epidemic diseases, especially plague and cholera. Its efficiency under such conditions could be tested by the experimental method.

ing rise and evening fall. A small pus sac was found posterior to the olecranon of the left arm. Aspiration yielded a few drops of lemon-colored serous liquid which, microscopically, contained numerous pus cells and a few capsulated rod-like bacteria. Later a larger pus sac was found on the dorsum of the left foot.

Sputum examination.—May 30, mucopurulent, containing a few blood cells; no tubercle bacilli.

Blood examination.—May 29, 21,000 leucocytes; June 3, 23,400 leucocytes; no parasites.

Urinalysis.—May 29, 1,021; alkaline, trace of albumin; no casts, many leucocytes. June 2, 1,019, alkaline, no albumin; no casts, few leucocytes; bile.

Diagnosis.—Pyemia; acute articular rheumatism, both elbows, knees, and ankles; suppurative inflammation of left elbow (type undetermined).

Treatment.—The treatment was essentially supporting and palliative.

About June 6 he developed a cutaneous eruption which was considered to be that of smallpox, since a smallpox patient had been removed from an adjoining bed seven days before the eruption appeared on Marshall. He was removed to the military smallpox hospital and died on the following morning. The body was sent to the city morgue marked "suspected smallpox case."

Incomplete autopsy No. 987 (about twenty-four hours after death).—No autopsy was requested, but Dr. W. E. Musgrave and Dr. W. R. Brinckerhoff, who happened to be at the morgue, noticed the peculiar cutaneous eruption and brought pieces of the skin to the laboratory for further examination. Large areas of skin surface were covered with the numerous, closely set vesicles of miliaria, and among these were numerous pustules of varying size. Some of these pustules were capped by a vesicle, which in some instances showed a central depression giving an appearance of umbilication.

None of us thought of human glanders until a miscroscopic examination showed that the pustules contained numerous bacteria morphologically resembling *Bacterium mallei*—rods about the length of and somewhat thicker than the tubercle bacillus, which stained irregularly with Löffler's methylene blue and lost the stain in Gram's method. (See Pl. IV, fig. 2.) No such bacteria were found in smears made from the abscess in the left ankle.

Bacteriologic examination.—Cultures were made on + 1 glyc-

erine agar slants from the contents of a pustule, and a bouillon emulsion of some of the contents of another was injected into the abdominal cavity of a male guinea pig. The cultures were kept at 35°–37°, and in twenty-four hours numerous discrete, barely visible, transparent colonies appeared. These were plainly visible in forty-eight hours, and a pure culture from one of them gave the biochemical characters which are described at the end of this bulletin as peculiar to *Bacterium mallei.*

The guinea pig showed distinct hyperemia and swelling of the testicles in forty-eight hours. It was chloroformed on the sixth day after inoculation. At autopsy a pin-head-sized, yellowish nodule was found at the point where the needle passed through the muscular wall of the abdomen. The testicles had reached the size of a large walnut and were fused together, and their greatly thickened tunica showed a number of yellowish, caseous foci. There was congestion of the inguinal glands, spleen, liver, and kidneys. No nodules were found in the organs of the peritoneal and thoracic cavities. *Bacterium mallei* was obtained in cultures from the testicles.

Histologic examination (skin pustule, Borrell's stain, kindness of Dr. Brinckerhoff).—A section through one of the larger pustules (about 4 millimeters in diameter) (see Pl. V) shows a densely infiltrated area in the skin and subcutaneous tissues. This inflammatory exudate lies chiefly between the muscular layer and the Malpighian cells of the epidermis. The epidermis is raised to a considerable distance above its level in the adjacent normal skin. At the point where it leaves its normal level the deeply pigmented cells of the rete malpighii are seen to be greatly elongated, and just before it reaches its greatest elevation there is a splitting away of the horny layer, which, continuing to a similar point on the opposite side, leaves a space which constitutes the vesicular portion of the pustule.

Under a high-power lens the contents of the vesicle is seen to be composed of degenerated polynuclear leucocytes and cells from the stratum granulosum, nuclear fragments, and a granular detritus which represents the products of cell degeneration and coagulated serum. Beneath this area the cells of the rete show marked infiltration and vacuolation, many of their nuclei staining but faintly or not at all. The deeper-infiltrated area is composed of a dense collection of more or less degenerated leucocytes and

erythrocytes, degenerated epithelial cells, and nuclear fragments—many of which seem to have coalesced to form irregular, deeply staining masses of chromatin. Here some islands of degenerating epithelial tissue, probably the remains of papillary pegs, may be seen. No giant cells are present. A very prominent feature is the widespread and extensive destruction of the nuclei of the fixed and infiltrating cells. This varies from simple vacuolation to complete karyorrhexis. A number of normal polynuclear cells may be seen with their protoplasm filled with rounded or rod-shaped nuclear fragments. The blood vessels of the subcutis show great congestion. The underlying muscular and glandular tissues appear normal. There are no signs of proliferation—everywhere those of degeneration.

CASE II.

This patient was a Filipino, 38 years of age, living in Santa Cruz, Manila. His previous history was obtained through the kindness of Dr. Edwin Shattuck, of Station G, Board of Health. It is as follows:

The case of Atanacio Castro was reported by Dr. Canuto Reyes, municipal physician, to Dr. R. L. Bartlett and myself. After seeing the case together we were unable to arrive at a diagnosis. The house was quarantined immediately and Dr. Brinckerhoff was notified of the peculiar eruption, with the result that he visited the case and secured a culture. About an hour after the culture was taken the patient died and the body was sent to the San Lazaro Morgue. The deceased was 38 years old, married, and a clerk by occupation. He was taken sick on the 2d of October, 1903, with chills and fever, tertian in character. In December of the same year he had an abscess on the posterior portion of the right leg, which was opened by the doctor who attended him. Again, in February of the present year, he had an abscess in the anterior and upper portion of the right side of the chest, which underwent resolution without operation. The fever and chills continued until (on June 27), an eruption appeared on the face and later on the chest and abdomen. On the third day of the eruption he died, namely, the day he was reported to Station G. The deceased had also suffered from articular pains. He was the owner of a stable where horses and vehicles were kept for hire. Furthermore, he lost several of his horses recently from glanders. Any credit for this report is due to Dr. Bartlett and Dr. Reyes.

Autopsy (about nine hours after death).—The body appears quite emaciated. A papulo-vesicular eruption is scattered over the skin surface (see Pl. IV, fig. 1). These lesions are most numerous on the face, back of the trunk and upper arms, buttocks and back

of the upper part of the thighs, and more scattered over the chest and the abdomen. They are not distributed regularly, but are grouped with intervening areas of comparatively free skin, and vary in size from 2 to 3 and 6 to 8 millimeters in diameter. The smaller ones appear as shotty papules, while the larger ones are distinctly vesicular. Several of these vesicles show depressed areas, which give them the appearance of being umbilicated, although more commonly their surfaces appear wrinkled. On section they are seen to be situated on a fairly well-defined, yellowish, firm nodular base, which extends into the subcutaneous tissue. The eruption on the face seems to be more advanced. On the forehead and cheeks the lesions appear as irregularly circular, raised, dark-red and purble-colored plaques about 1 to 1.5 centimeters in diameter (see Pl. III, fig. 2). Some of these are rounded and nodular, others flat with depressed center and raised edges. A few present a reddish-yellow, ulcerated center surrounded by raised edges. Two of these nodular plaques occur beneath the skin of the scalp, just above the upper margin of the forehead.

The tissues just to the right of the nose are so swollen as to close the right eye, which itself does not seem to be affected. A section through these swollen tissues reveals many discrete and confluent, yellowish and grayish, caseous foci, which are surrounded by congested and necrotic tissue and extend to the depth of about an inch from the skin surface. The alæ nasi are thickened by similar nodules, and one can be seen on the mucous membrane of the upper lip, just to the left of the nasal fossa. A dirty, purulent discharge escapes from the nostrils. Upon opening the nares, the mucous membrane, especially of the right one, is seen to be ulcerated. The ulcerations cover most of the mucous surface of the floor and walls of this nostril and extend upon the inferior turbinated bone. (See Pl. III, fig. 1.) Small, grayish or yellowish nodules may be seen projecting from the ulcerated surfaces. The right nostril is not affected to such a marked degree. The ethmoidal cells are filled with purulent matter. The right clavicle is much thicker than the left, but nothing of note is seen on section.

The thoracic cavity contains no fluid. Both lungs are bound to the thoracic walls by firm, fibrous strings of adhesions. The lungs are emphysematous anteriorly, and posteriorly show considerable hypostatic congestion. On palpation small nodules can be felt

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beneath the visceral pleura, which on section appear as pea-sized or smaller, circumscribed, grayish-yellow areas of a confluent tubercular structure. (See Pl. III, fig. 3.) None of them are capsulated or caseated, but some are surrounded by an irregular, reddish area of pneumonic consolidation. They seem to be limited to the pleural surface.

The bronchial glands appear normal on section. The trachea and bronchi are slightly congested and covered by a muco-purulent secretion. The heart muscle is rather pale on section, but otherwise the organ appears normal. The mucous membrane of the esophagus shows hyperplasia of the solitary follicles.

The liver is of about normal size, soft, and on section its markings are indistinct. The spleen is somewhat enlarged, soft, and on section its pulp is diffluent. The kidneys are slightly enlarged, their capsules strip readily, and on section the cut surface is yellowish-white in color and the corticle and medullary markings are very indistinct. The stomach and intestines were not opened. No further examination was made.

Bacteriologic examination.—Dr. W. R. Brinckerhoff, visiting the case shortly before death, thought that it might be another one of human glanders and brought some of the fluid contents of the vesicle to the laboratory. The cornea of a rabbit was scarified and inoculated with a small quantity of the fluid in order to exclude the existence of the cytoryctes of smallpox. Coverslips of the secretion were stained with Löffler's methylene blue and examined by Dr. Brinckerhoff. They revealed quite a number of organisms resembling those occurring in Case I. (See Pl. IV, fig. 2.) Glycerin agar slant cultures showed a number of small, white, discrete colonies, after incubation for twenty-four hours at 35°–37°. In forty-eight hours these had produced a golden-yellow pigment. Microscopically they were composed of cocci, which were not studied culturally. The twenty-four-hour cultures also showed numerous barely visible, transparent colonies lying between the larger ones. In forty-eight hours the former became quite visible and in pure cultures gave the biochemical reactions which have been described as characteristic of *Bacterium mallei*. The rabbit whose cornea was scarified died in forty-eight hours. No autopsy was made. It is possible that it died of staphylococcus infection, for the fluid had been collected without precautions against contamination.

Post-mortem, coverslip preparations and cultures were made from the pustules after searing the surface of the skin, and showed that at least the vesicles examined contained *Bacterium mallei* in pure culture. Similar organisms were found in the smears from the nasal discharge and the lung nodules.

A male guinea pig (No. 608) was inoculated intraperitoneally with a small portion of the contents of a vesicle. In five days the testicles had swollen to the size of a walnut and were intensely hyperemic and tender. The animal was chloroformed and at autopsy the only visible lesions were in the testicles, where numerous yellowish, caseous nodules were found scattered about, especially throughout the inferior and thickened portions of the tunica vaginalis. A pure culture of *Bacterium mallei* was obtained from these lesions.

Histologic examination (tissues fixed in Zenker's fluid, imbedded in paraffin and stained with hematoxlyin and eosin)—Small skin pustule (about 2.5 millimeters in diameter) : The histologic changes in the subcutis and lower layers of the cutis are similar to those described in Case I. The process is not, however, so far advanced, for, though karyorrhexis is widespread and prominent, it is not so marked in the upper layers of the cutis, where the chief changes are a loss in the staining power of the nuclei and a general vacuolation of the cell protoplasm. The stratum corneum has not been split off and consequently the pustule is not covered by a vesicle.

Lung: A section through one of the subpleural nodules (about 3 millimeters in diameter) shows, under a low magnification, an irregular area of consolidation characterized by intense infiltration of the pulmonary alveoli and marked congestion of the blood vessels of the alveolar walls and of the pleura covering the affected area.

Under a higher power the contents of the alveoli is seen to be composed cheifly of polynuclear and transitional leucocytes, a few lymphocytes, pigment-carrying cells, and a few large cells which lie, for the most part, near the alveolar walls and resemble desquamated endothelial cells. It is apparent that many of the cells in this area are undergoing degeneration and karyorrhexis, but not to so marked an extent as in the skin pustule of Case I. This area is surrounded by pulmonary tissue which shows intense congestion and in which the alveoli are filled, for the most part, with extravasated blood, granules and threads of fibrin, desquamated endothelial cells and a few polynuclear and transitional leucocytes. Deeper within the section the alveoli appear normal.

In another field of the section several small foci of infiltration may be seen, situated at some distance beneath the pleural surface and each is about the size of a single air cell. Their contents is composed of cells similar to those found in the larger focus, but karyorrhexis is not so marked a feature. One of these appears to have ruptured into an adjoining alveolus, and such a process may indicate the histogenesis of the larger foci. All the foci appear to be recent ones, and there are no signs of proliferation or encapsulation. No giant cells are to be seen.

Spleen: This organ presents nothing noteworthy, excepting intense congestion.

Liver: There are no inflammatory foci, but throughout the section the hepatic cells are granular and most of the nuclei stain poorly or not at all.

Kidneys: The blood vessels and glomeruli are congested throughout. The cells lining the tubules are granular and many of their nuclei stain poorly. No inflammatory foci are seen.

Demonstration of Bacterium mallei in sections.—It is generally stated that the micro-organisms are stained in the tissues only with the greatest difficulty. They were demonstrated very plainly in sections of the lung nodules and skin pustules by Dr. Paul G. Woolley, Director of the Serum Institute, by the following method:

Paraffin sections of tissue fixed in Zenker:
Remove paraffin with xylol.
Absolute alcohol.
95 per cent alcohol.
80 per cent alcohol.
Water.
Tincture of iodin till well browned.
80 per cent alcohol.
Water.
Immerse in Unna's alkaline methylene blue until purple.
Absolute alcohol a second.
Xylol. Wash and then immerse for ten minutes.
Balsam.

Unna's alkaline methylene blue:
Methylene blue, 1 gram.
Potassium carbonate, 1 gram.
Water, 100 grams.

Steam in Arnold sterilizer for one-half hour to one hour. An essential to success is that the dehydration with absolute alcohol must be performed as rapidly as possible, a second or so being quite sufficient.

These two cases, although somewhat incompletely reported, are very interesting in that they illustrate the tendency to diagnose human glanders as some other disease. In both of them, when the cutaneous eruption occurred, a diagnosis of smallpox was made. The prevalence of glanders in Manila and the filthy, negligent habits of some of the population would lead one to suspect that the disease is transmitted to man more often than past statistics will show.[1] It would be interesting to know just to what extent "failures in vaccination" could be traced to such cases.

From the histologic examination it seems to be quite evident that the process is essentially a degenerative one, in which there is a total absence of any signs of proliferation. In this we can support the contention of Tedschi, of various French writers, and of Wright and Councilman that the glanders nodule is in no way related histogenetically to the tubercle, as originally held by Baumgarten.

It is altogether probable that such cases would have been overlooked for some time to come if it were not for the unusual interest the Harvard Commission, working in this laboratory (consisting of Drs. Brinckerhoff and Tyzzer), has taken in all cases in any way resembling or diagnosed as smallpox.

SOME OBSERVATIONS ON THE BACTERIOLOGY AND POLYMORPHISM OF BACTERIUM MALLEI.

The following is a summary of the biochemical characters of *Bacterium mallei*, as noted in the study of several cultures from equine and human sources.

Many pathogenic organisms, when transplanted from the animal body to artificial media, grow luxuriantly from the very start. On the other hand, certain bacteria seem to require a little time to accommodate themselves to such a change of environment; and all the cultures of *Bacterium mallei* that I have studied have shown a peculiar tardiness in the appearance of the growth, all primary transplants from the tissues upon nutrient agar or glycerin agar showing no growth, or at the most, a barely visible one at the end of the first twenty-four hours at body temperature. However, when the step from parasitism to saprophytism has been taken,

[1] Since the above was written a third human case has been encountered. This case occurred in a Filipino policeman who entered the Civil Hospital, where he was treated for pyæmia. The necropsy was perfurmed by Dr. R. P. Strong, who kindly turned the material over to me for further study.

all successive transplants grow more readily until the luxuriance of the growth reaches a maximum. A similar delay in the appearance of the primary growth has often been observed in certain cultures of plague, dysentery, and other bacteria.

I have not experienced the difficulty, which is mentioned by some authors, in keeping cultures of *Bacterium mallei* alive. Possibly this is because the cultivations were made upon media of very nearly the same reaction. Cultures isolated over nine months ago are still alive. They have been transplanted upon glycerine agar at intervals of about three months and kept in the ice chest.

BIOCHEMICAL CHARACTERS (AT 35°–37°).

+1 Agar.[1]—A moist, whitish, translucent streak with even edges appears in twenty-four hours. The growth reaches its maximum in about three days, when the edges become slightly crenated. The growth is quite viscous when touched with the platinum needle. There is no further change during nine or ten days.

+1 agar with 3 per cent glycerin.—The growth is slightly more rapid than on +1 agar, and the luxuriance of the growth increases steadily for five to six days. Saprophytic cultures may spread over the whole surface of a slant. The growth becomes smooth, shining, light brown in color, and quite viscous. Some old cultures present large, fan-like crenations along their edges.

+1 agar with 3 per cent sodium chloride.—A scarcely visible growth appears in twenty-four hours. In forty-eight hours a very slight, dryish, granular growth may be seen by reflected light. There is no further increase and the culture is found to be dead to further transplantation after a few days.

+1 sugar-free bouillon.—The growth occurs as a uniform cloudiness. A viscous sediment collects during eight or nine days. No indol is produced in ten days.

1 per cent peptone (Witte's) solution.—A faint turbidity, accompanied by the formation of a slight sediment is produced in twenty-four hours, after which there is not much increase. No indol is produced in ten days.

[1]The culture media was prepared according to the recommendations of the American committee, excepting that the reaction to phenolpthalein was adjusted by the addition of normal sodium hydroxide alone. +1 means 1 per cent acid with phenolphthalein as an indicator. (See the Reports and Papers of the American Public Health Association, 1898, 23, 60.)

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Litmus milk.—The changes produced have varied on different occasions. Usually no visible change is produced during two or three days. In some instances slow acid production has subsequently appeared, with coagulation on the sixth or seventh day. The cultures isolated from the human cases only reduced the litmus in the milk and produced no further change during eight days' observation. Control tubes remained unchanged. (This variation may be due, possibly, to changes produced in the milk before sterilization.)

—*1 bouillon.*—There is no sign of growth in the upper layers of the fluid, but a slight, stringy precipitate rises if the test tube is shaken.

+*1 gelatin (containing muscle sugar) at 18° to 22°.*—There is nothing characteristic about the growth. A slight, whitish growth appears along the stab and upon the surface. No liquefaction occurs in ten days.

Potato (unneutralized, with a reaction of about +2).—A luxuriant, moist, shining, yellowish-white growth appears in twenty-four hours. This is quite viscous and in a few days takes on a yellowish-brown color. (The growth is well described as resembling a streak of honey; but I have not noted any decided pigment production during nine or ten days' growth, such as is described by some observers.)

+*1 glucose, lactose, and saccharose bouillon in the fermentation tube.*—Only one culture was tested (human glanders, Case I, skin pustule). In this case good growth occurred in the bulb and open neck of the fermentation tube, while the closed arm in each instance remained clear. No test for changes in reaction were made.

Morphology and polymorphism.—The morphology of the organism varies considerably upon media of different reaction and composition, as illustrated in Plate VI. Very minute rods usually appear on +1 agar. On +1 glycerin agar and on potato the form is more like that which is seen in preparations from the animal body. On +1 agar containing 3 per cent salt (Cross and Blackwell's table salt, which probably contained traces of nitrates) irregular, curved, and sinuous rods, with rounded ends or irregular, branching forms appeared in one culture. Most of these rods, when taken from a forty-eight-hour culture, stained evenly, though some showed irregular vacuolation of the protoplasm. Clubbed forms were seen. These involution forms were from 3.7 μ–10 μ

in length and about 1.5 μ thick. On other trials, where chemically pure sodium chloride was used, such large branching forms did not appear, but the production of large, sinuous, and clubbed rods was quite noticeable.

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DESCRIPTION OF PLATES.

PLATE I. (Photograph, natural size, by C. H. Martin, photographer of e Bureau.) Showing a large, confluent, nodular, and ulcerated area on the nasal septum of a horse which died of glanders at the quartermaster's corral in December, 1903. The submaxillary glands were involved and a number of subpleural nodules were found in the lungs. These nodules had caseous centers and were surrounded by well-defined gelatinous capsules from which the contents could be enucleated. Deeper within the lungs, large, white connective tissue tumors with central areas of softening were found. A couple of pea-sized nodules were seen in the spleen on section.

Bacterium mallei was found in stained preparations from the nasal ulcers and lung nodules and isolated culturally and by the Strauss method. Cultures were obtained from some of the lung nodules which appeared sterile upon microscopical examination.

II. (Photograph, natural size, by C. H. Martin.) Showing another type of ulceration—numerous smaller areas of confluent and ulcerated nodules—on the nasal septum of a horse which also died of glanders at the quartermaster's corral in December, 1903. *Bacterium mallei* found microscopically and by culture.

III. Fig. 1 (painted by T. Espinosa, artist of the laboratory), illustrating the ulceration in the right nostril of Case II, human glanders.

Fig. 2 (original) shows the appearance of the ulcerated plaques on the forehead of Case II, human glanders.

Fig. 3 (original) shows a section through a couple of lung nodules occurring in Case II, human glanders.

IV. Fig. 1 (photograph, natural size, by Roman Mercado, assistant photographer of the Bureau,) shows the character of the cutaneous eruption in Case II, human glanders. The variation in size and general lack of umbilication are noteworthy points in differentiating it from that of smallpox. On account of shrinkage the skin and pustules appear more wrinkled than they did before removal from the body.

Fig. 2 (photomicrograph, about X 880, by C. H. Martin) shows the characteristic morphology of *Bacterium mallei*, as seen in a coverslip preparation from one of the skin pustules of human glanders (Case II) (Carbol fuchsin 1.10). The shape, size, and the irregular staining of the rods, together with the evident nuclear fragmentation, is quite pathognomonic.

V. (Photomicrograph) low magnification, by C. H. Martin.) Shows a section through one of the skin pustules of human glanders (Case I); Borrell's stain (kindness of Dr. Brinckerhoff).

VI. (For description see Pl. VI.)

VII. (After Kitt.) Illustrating the characteristic stellate cicatrices sometimes seen on the nasal mucous membrane in chronic glanders.

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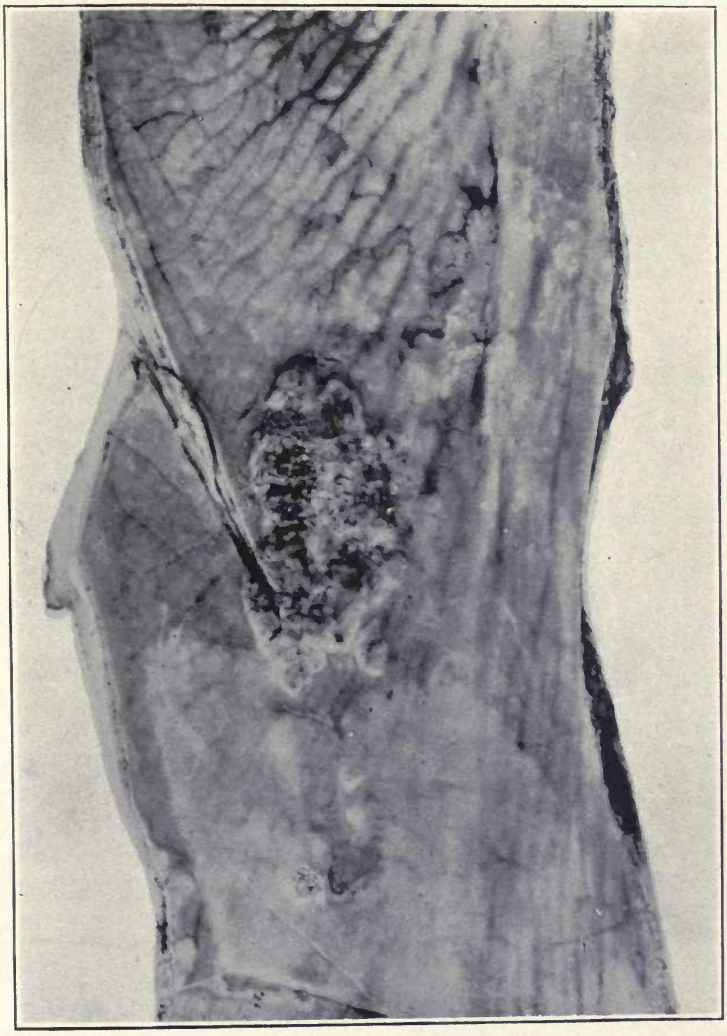

PLATE I.

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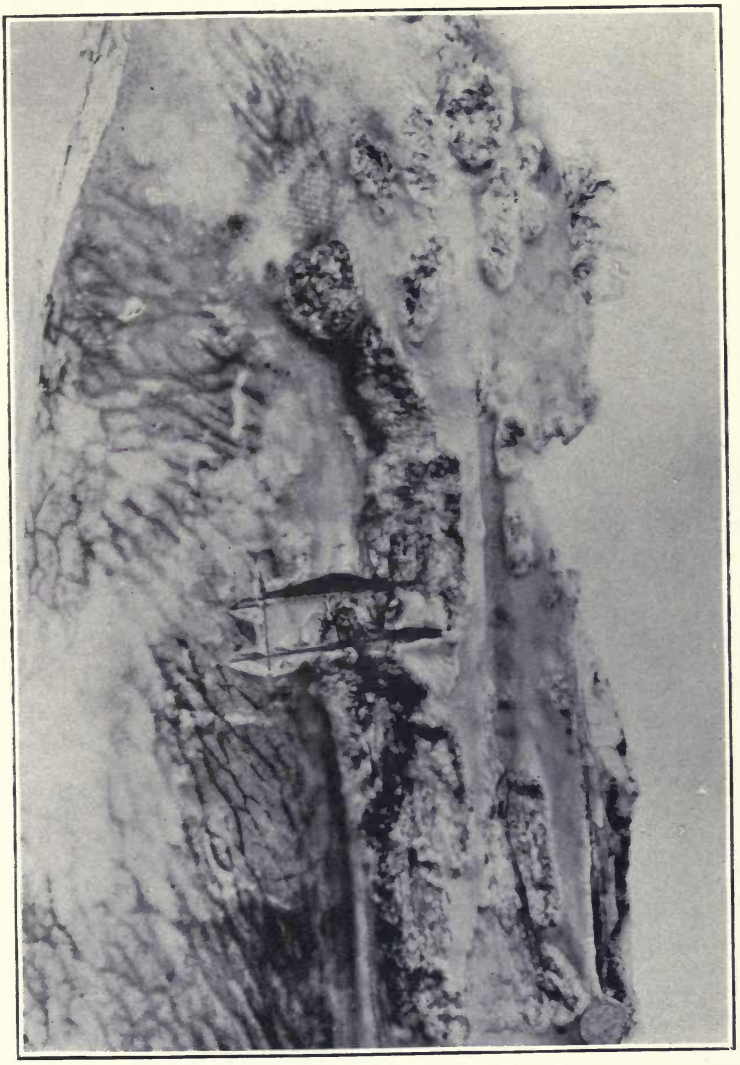

PLATE II.

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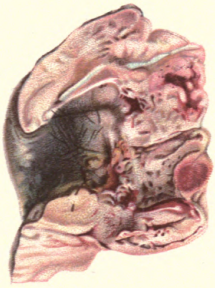

Fig. 1

Fig. 2

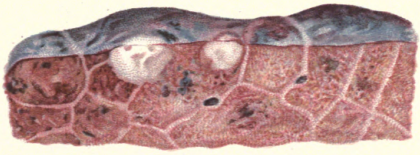

Fig. 3

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FIG. 1.

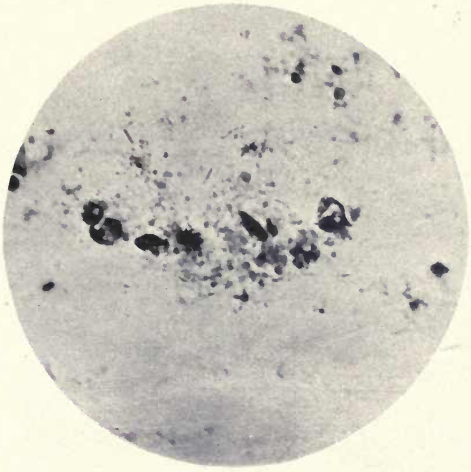

FIG. 2.

PLATE IV.

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PLATE V.

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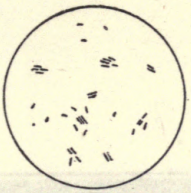

From three days' growth on +1 agar slant at 35°–37°.

From three days' growth on +1 agar with 3 per cent glycerine at 35°–37°.

From three days' growth on potato (unneutralized), 35°–37°.

From two days' growth on +1 agar containing 3 per cent salt at 35°–37°.

From the same salt agar culture after nine days' growth at 35°–37°.

PLATE VI

PLATE VI

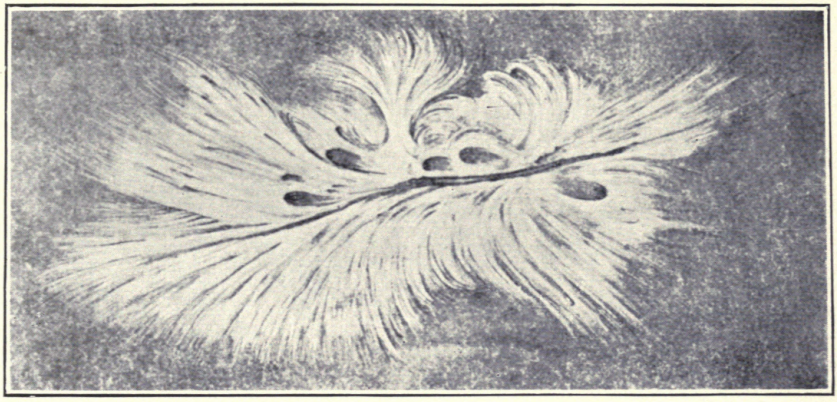

PLATE VII.

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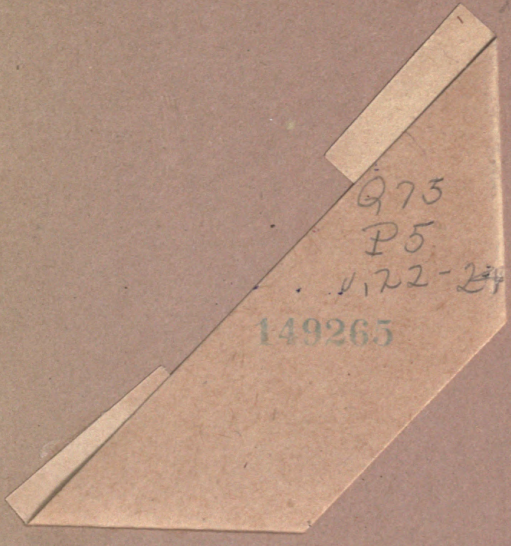
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