

MELIOIDOSIS AND ITS RELATION TO GLANDERS.

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(From the Institute for Medical Research, Federated Malay States.)

(With Plate X.)

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1. INTRODUCTION.

MELIOIDOSIS bears such a striking resemblance to glanders, that Colonel Whitmore, who was the first to recognise this disease, described it under the title of "A glanders-like disease occurring in Rangoon" (Whitmore, 1913). When he first met with the disease in the post-mortem room, he thought that he was dealing with glanders. It was not until he discovered that the lesions were associated with the presence of a strange organism that he concluded that he had encountered a new disease.

The pathological features of melioidosis and glanders are almost, if not quite, identical. In both diseases there are pyaemic nodules which have a tendency to caseate in some organs and to suppurate in others. In both diseases the minute structure of the nodules is the same. McFadyean states that the two distinguishing characteristics of the glanders nodule are, first, the peculiar degeneration, or chromatotaxis, which occurs in the centre of it, and secondly, its slight tendency to peripheral extension. These features are equally characteristic of the melioidosis nodule, and it is difficult if not impossible to distinguish one disease from the other by means of inspection with the naked eye or by the examination of sections under a microscope.

The two diseases resemble each other clinically almost as much as they do in their morbid anatomy. The first case of melioidosis to be observed throughout its course was under the care of Major Knapp in the gaol at

Rangoon. The symptoms were like those of glanders, and it was only when a bacteriological investigation was made, after death, that the true nature of the malady was disclosed (Whitmore, 1913).

Knapp (1915) suggested that the new disease might be distinguished from glanders by the absence of skin lesions, but these occur in both diseases; bullae and pustules have been present in several of the cases which we have seen in Kuala Lumpur. Pustular rashes, abscesses in the subcutaneous tissue, in the muscles and in the bones are features of the later stages of melioidosis as well as of glanders. Both diseases may resemble malaria, acute rheumatism, enteric fever or tuberculosis and, in the early stages, diagnosis may be impossible.

On comparing our experience of melioidosis with the text-book descriptions of human glanders, we find that there is a difference in the usual course of the two diseases. Melioidosis is more often acutely fatal. The majority of our patients died within a period of ten days after being attacked, and nearly all Whitmore's cases must have died very shortly after the onset of the disease, because they were either dying or already dead when they were brought to the hospital.

When animals become infected, nasal discharge is a common symptom in both melioidosis and glanders; nodules are often present in the nasal septum, and the lungs are almost invariably diseased.

Chronic melioidosis is rare in man and it has not been possible to apply the mallein test in more than one case. In this instance it produced a positive reaction. We obtained a supply of mallein through the kindness of the Director of the Geneeskundig-Laboratorium at Batavia. This was diluted with nine parts of salt-solution and 0.2 c.c. were injected into the skin of the forearm of a patient who had been suffering from melioidosis for two years. In less than two hours his temperature had risen to 100.8° F. On the following day, eighteen hours after the inoculation, the temperature was 99° F. and there was a large, red, diffuse, tender swelling, at the site of inoculation, which measured 10 by 6 cm. On the third day, forty-two hours after inoculation, the temperature was 99.6° F.; the swelling was larger and the patient complained that pain had kept him awake during the previous night. The fever and swelling then subsided and, within ninety-six hours of the time at which the injection was given, all signs had disappeared. Four controls were inoculated with entirely negative results.

Mallein tests in laboratory animals did not prove satisfactory. Two rabbits, which had been immunised with killed cultures of a strain of *B. mallei* obtained from Java, were inoculated with 0.02 c.c. of mallein, and in each of them there was a slight reaction in the form of a small, red papule which lasted for about three days. Another rabbit which had been immunised with a culture of *B. whitmori*, the causative organism of melioidosis, gave a similar result. None of these reactions was nearly so definite and pronounced as in the human case.

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A guinea-pig immunised with killed cultures of *B. mallei*, Java, did not react to mallein. Two guinea-pigs with abscesses resulting from injections of living *B. mallei* gave no reaction. Three guinea-pigs with large abscesses due to injections of *B. whitmori* failed to react with mallein.

The intimate relationship of melioidosis and glanders is clearly demonstrated by the presence of agglutinins for *B. mallei* in the blood of persons suffering from chronic melioidosis. The blood of the man, Ragaviah, who gave the positive mallein reaction, agglutinated *B. whitmori* (cultivated from his own lesions) in a dilution of 1 in 4000. Tested with three strains of *B. mallei*, it agglutinated two of them (strains "Muktesar" and "Java") up to 100 per cent. and the other strain "Minett" up to 10 per cent. of the full titre. In addition, one of these strains, *B. mallei* "Muktesar," absorbed the agglutinins for the homologous *B. whitmori*.

As we have seen, there is little or no difference in the morbid anatomy of glanders and melioidosis, and the difference in their clinical course lies more in the greater pathogenicity of the latter infection than in its fundamental characters. Their epizootology, however, appears to be quite distinct; both are primarily diseases of animals and rarely affect man; but while glanders is an equine disease, melioidosis is a disease of rodents (Stanton and Fletcher, 1921). There are very few horses in the Federated Malay States and, in those cases of melioidosis where it has been possible to investigate this point, there has been no opportunity of infection from these animals.

Apart from other differences in the two diseases, it was because the organisms which caused them appeared so totally unlike one another on cultivation outside the body that Whitmore decided that his cases were examples of a new disease caused by a new bacillus. He (Whitmore, 1913) stated that the cultural and other characteristics, upon which he relied for distinguishing this bacillus from other pathogenic bacteria, were as follows:

1. Rapid and luxuriant growth upon ordinary peptone agar.
2. The wrinkling which occurs so early in the growth upon glycerine agar.
3. The pellicle formation at the surface of broth cultures.
4. The appearance of gelatine stab cultures at the end of the third day, and after one week's growth.
5. The curious, tangled masses of long filamentous bacilli found in cultures upon salt agar.
6. The active serpentine motility of the bacilli in young cultures and its disappearance as the cultures age.

In order to determine if these criteria are fulfilled by the bacillus of glanders as well as by Whitmore's organism, we obtained five strains of *B. mallei* which we compared by means of cultural and serological tests with fourteen strains of *B. whitmori*.

The sources of the five cultures of the glanders bacillus were as follows:

- (1) *B. mallei*, Java. This strain was kindly sent to us by the Director of the Geneeskundig-Laboratorium at Batavia, in Java, on August 8, 1922.

It had been isolated from the lungs of a country-bred pony, more than a year previously, and had been passed through a number of guinea-pigs.

(2) *B. mallei*, Muktesar. A culture of this organism, from the Imperial Bacteriological Laboratory at Muktesar, in India, was received in September, 1922. Dr J. V. Edwards, the bacteriologist, kindly informed us that this strain was isolated from a country-bred pony and had been maintained for a long time in culture and by passage through guinea-pigs.

(3) *B. mallei*, Minett. This strain, and the two which follow, are from the National Collection of type cultures, and were supplied through the kindness of the Director of the Lister Institute. The Minett strain was isolated from the lung of a horse at the Royal Veterinary College in 1918.

(4) *B. mallei*, Egypt, was isolated in November, 1922, by Captain F. C. Minett, in Egypt, from a case of cutaneous glanders in a horse.

(5) *B. mallei* A. is the stock strain of the glanders bacillus at the Lister Institute.

The origin of the fourteen strains of *B. whitmori* was as follows: They comprised ten which had been isolated from human cases and four cultivated from animals naturally infected with melioidosis. One of the human strains, *Finlayson*, was isolated from the lung of a patient in Singapore. The other nine were obtained from cases in Kuala Lumpur. Four of these nine cultures, namely *Govindasamy*, *Pavaday*, *Sellen* and *Vengkatten*, were grown from spleens; two, *Mananay* and *Warton*, were from livers; *Velu* from a lung; the two cultures *Ragaviah* (corrugated) and *Ragaviah* (mucoid) were both isolated from a loopful of pus taken from an abscess in the leg of a patient suffering from chronic melioidosis.

The origin of the four animal strains was as follows: The strain known as *Cat* was cultivated from the spleen of a domestic cat which was brought to the laboratory with melioidosis contracted under normal conditions; probably from eating an infected rat. The strain *Jacques's rat* was isolated from the caseous lungs of a rat which was found dead from melioidosis in the house of a European officer, five miles distant from this laboratory. *Rabbit 45* (corrugated) was obtained from the spleen of a rabbit which had become infected in the animal-house attached to this Institute. *Rabbit 45* (mucoid) was cultivated from the caseating tunica vaginalis of the same animal.

We have adopted the two strains *Ragaviah* (corrugated) and *Ragaviah* (mucoid) as our standard cultures of *B. whitmori*. As mentioned already, both of them were isolated from an abscess in the leg. We have related elsewhere (Stanton and Fletcher, 1921) that *B. whitmori* occurs in two forms; first the more common variety which grows as an opaque culture with a corrugated surface on glycerine agar and, secondly, a mucoid variety which forms a smooth, translucent film on the same medium (see Pl. X). The mucoid variety of *B. whitmori* bears the same relationship to the ordinary corrugated form as the mucoid variety of *B. paratyphosus* bears to the

ordinary form of that organism (Fletcher, 1920). Corrugated colonies can be grown from a mucoid culture, and we have proved the identity of the two types by agglutination and absorption tests. If peptone water is inoculated with the mucoid culture of the "Ragaviah" strain of *B. whitmori* employed in these experiments, and after ten days' incubation at 37° C. is plated out on agar, then about 10 per cent. of the colonies on the plate are of the corrugated type; the corrugated colonies are "thrown off" by the mucoid form and are derived from it. The fourteen strains which were used in the following experiments were all of the common corrugated form except *Ragaviah* (mucoid) and *Rabbit 45* (mucoid).

2. THE CULTIVATION OF *B. WHITMORI* AND *B. MALLEI*.

In peptone water the mucoid strains of *B. whitmori* are indistinguishable from glanders cultures, except that growth is more vigorous in the former; but the appearance of the corrugated form of *B. whitmori* is quite different from its own mucoid form and from *B. mallei*. The corrugated strain forms a film on the surface of the medium which becomes a thick, wrinkled pellicle in the course of two or three days. The strains of *B. mallei* which we examined did not possess this property. The culture from Muktesar grows as a film on the surface of peptone water, but this film does not become thick or wrinkled. The other strains of the glanders bacillus produce ropy deposits in the depth of the medium, and the upper part becomes clear. There are no obvious differences in the morphology of the organisms as seen under the microscope in a hanging drop; but the cultures of *B. whitmori* are actively motile and thus they can be distinguished at once from the strains of *B. mallei*, none of which are motile even after daily subculture in liquid media for a period of three weeks. Indol is not formed in peptone water, either by *B. whitmori* or *B. mallei*.

The appearance of cultures of the corrugated form of *B. whitmori* on solid laboratory media is entirely different from that of *B. mallei*. On ordinary agar, *B. whitmori* is white and opaque instead of being slimy and semi-transparent. On glycerine agar it is wrinkled and corrugated, like an old culture of tubercle bacilli, whereas *B. mallei* is smooth and shining (see Pl. X). Cultures of the mucoid form of *B. whitmori* on solid media are not unlike *B. mallei*, but the growth of the former is more vigorous and the diagnosis can be speedily settled by inoculating a tube of peptone water and examining the culture for motility eighteen hours later.

The growth of the corrugated form of *B. whitmori* on potato is quite different from all the other cultures investigated. It is a dull, dirty white, not shining and yellow like *B. mallei*. The mucoid forms of *B. whitmori* on potato are indistinguishable from *B. mallei*.

The difference between *B. whitmori* and *B. mallei* in their action on carbohydrates is one of degree. *B. whitmori* ferments glucose; the surface of glucose peptone-water becomes pink, in about thirty-six hours, when Andrade's indicator is employed. The colour soon spreads through the medium, reaching

the Durham's gas-tube last of all. Bleaching of the indicator commences on the fourth or fifth day and proceeds in the same order, being complete by about the tenth day. Mannite and dulcitol show a pink tinge at the surface in four or five days, but the process goes no further. Lactose and saccharose are not fermented by strains which have been subcultured repeatedly in the laboratory. No gas is produced in any medium.

The fermentation reactions of the bacillus are more vigorous when it is first isolated from the body. The first subculture of the strain *Warton*, which was isolated from a human liver in December, 1923, fermented glucose and saccharose in twenty-four hours and, in seventy-two hours, it fermented lactose, mannite and dulcitol as well. Two months later, in February, 1924, it still fermented glucose, mannite and dulcitol but not lactose or saccharose. In March, it fermented glucose as before, but its action on mannite and dulcitol had become exceedingly feeble.

All the strains of *B. mallei*—Muktesar, Java, Minett, Mallei A. and Egypt—ferment glucose in seven to thirty days. The medium remains red; the indicator is not bleached, as in the case of *B. whitmori*. Lactose, saccharose, mannite and dulcitol are not fermented by *B. mallei*.

B. whitmori has a more destructive action on milk than *B. mallei*, but here again the difference is only one of degree. Litmus milk inoculated with *B. whitmori* becomes acid in three days. In five days it separates into a fatty curd on the surface, an intermediate layer of dirty, brown whey, and a granular, buff-coloured deposit at the bottom of the tube.

Milk inoculated with *B. mallei* becomes acid in about the same length of time, but it does not clot until the tenth day. The clot is bright pink above, and white below. On the surface of the medium there is a bright, pink, creamy layer. The clot is not digested, but it contracts and exudes a colourless whey.

Gelatine is liquefied very rapidly by *B. whitmori* and very slowly by *B. mallei*. All the strains of *B. whitmori* which we examined commenced to liquefy gelatine on the fourth or fifth day, at a temperature of 20° C.; but none of the *B. mallei* cultures grew well at this temperature and none liquefied gelatine. At 37° C. gelatine is completely liquefied by *B. whitmori* in four days. That is to say, it is digested and does not become solid when placed in the refrigerator. Liquefaction, at this temperature, occurred on the thirty-sixth day with *B. mallei*, Minett; on the fortieth day with *B. mallei*, Muktesar, and on the twenty-sixth with *B. mallei*, Java.

It is impossible to distinguish *B. whitmori* from *B. mallei*, with certainty, by the examination of stained films. Formerly we considered that bipolar staining with Leishman's stain was a distinguishing feature of *B. whitmori*, but, in preparations made from peptone-water cultures, we found bipolar organisms in all three cultures of *B. mallei* as well as in those of *B. whitmori*.

In films prepared from cultures on ordinary agar, stained with Leishman's stain, many strains of the corrugated form of *B. whitmori* consist almost

entirely of deeply staining, bipolar organisms. There are also some bipolar bacilli in the cultures of *B. mallei* from Muktesar and Java, but they are not so numerous, nor do they stain so deeply. The Muktesar strain contains long hyphae, some as long as 70 micromillimetres, which break up into bipolar-staining bacilli. These hyphae are particularly remarkable in the ropy deposit which is found at the bottom of glucose-broth tubes, inoculated with *B. mallei*. In the case of the Java bacillus some of these long forms are beaded and branched like moulds.

Whitmore lays stress upon the long strings and chains formed by the bacillus on 2 per cent. salt-agar. This phenomenon is more common with the corrugated than with the mucoid form, but it cannot be relied upon, because it is not a constant feature of *B. whitmori*, and also because *B. mallei* sometimes grows in a similar fashion.

3. MELIOIDOSIS IN ANIMALS.

Melioidosis has been present among our stock of laboratory animals, first as an epidemic and later in sporadic form, for nearly eleven years. An outbreak which attacked the rabbits and guinea-pigs occurred at the end of 1913. A large number died and, in addition, a couple of rats which had been caged for experimental purposes became infected and succumbed to the disease. The earliest symptom in rabbits and guinea-pigs was a white milky discharge from the eyes and nose; within a few days the breathing became difficult and the animal died. Every case was fatal. The appearance found post-mortem depended very largely upon the duration of the illness.

In animals which had died very quickly from acute septicaemia, the only visible signs of disease, apart from the milky discharge about the eyes and nose, were congestion of the nasal mucous membrane, trachea and lungs, and a few, yellow, miliary nodules on the nasal septum. As a rule the bladder was noticeably distended with urine; sometimes this distension was so great that the top of the bladder reached the costal margin, and in one rabbit, of small size, the contents measured more than half a pint.

When the course of the disease was slower the lungs contained minute, caseous nodules, like miliary tubercles which consisted of a bronchiole and its surrounding alveoli filled with nuclear debris. In animals which lived still longer the lungs were consolidated, the swollen and detached epithelial lining blocked the bronchi, in places all alveolar structure was lost, while here and there small haemorrhages were seen. In the two rats, where the course of the disease was far less acute than in rabbits and guinea-pigs, the miliary nodules had spread and coalesced until the thorax was filled with a caseous mass firmly adherent to the thoracic walls. Very little lung tissue remained which was not caseous; in the caseous mass the heart was embedded, and the oesophagus behind it was studded with minute caseating nodules.

The characteristic lesion of the disease is a small, yellowish, caseous nodule. These nodules were found in almost every part or organ of the body and they

sometimes coalesced to form large caseous masses. *B. whitmori* could be cultivated from them in whatever situation they occurred and it was usually found in films made from the smaller nodules though not often present in very large numbers.

Histologically, as in several other respects, melioidosis resembles glanders. Both diseases are characterised by the formation of nodules which commence as small collections of polynuclear cells; these subsequently necrose, but their chromatin persists and retains its affinity for nuclear stains. This persistence of chromatin fragments is so striking a feature of the glanders nodule that Unna coined a special name for it, "chromatotaxis."

The spleens of our naturally infected laboratory animals were always enlarged. Except in very acute septicaemic cases they were speckled, like the spleens of plague rats, with small nodules of focal necrosis composed of broken down nuclei embedded in necrotic caseous material. Melioidosis usually runs a more chronic course in native rats (*M. griseiventer*) than in guinea-pigs and rabbits. In rats which have died from melioidosis the spleen sometimes contains large caseous nodules very like those found in the spleen of a rat with resolving plague.

The hearts of two of the rabbits contained specific lesions in the form of caseous nodules, the size of split-peas, adherent to the endocardium in the left auricle. These nodules consisted of fibrin and nuclear debris invaded by small cells and blood vessels. Fluid was sometimes found in the pericardium and, in a few guinea-pigs, the sac was full of caseous material.

In some cases the peritoneum was covered with small tubercles, like the tubercles of miliary tuberculosis, and the great omentum was rolled up and studded with yellow caseous nodules. The liver was affected in about one-eighth of the animals. In one of the rabbits there were nodules in the kidney. The disease frequently attacked the testis and epididymis. The testis was fixed in the scrotum by adhesions, while the epididymis and the tunica vaginalis were represented by a mass of caseous nodules with the usual histological characters. The lymphatic glands in the regions of the jaw, neck and axillae were sometimes enlarged and haemorrhagic, with caseous nodules in their substance.

4. THE INOCULATION OF ANIMALS WITH *B. WHITMORI*.

We attempted to reproduce the disease by inoculating laboratory animals with the organisms isolated from rodents which had become infected naturally, and numbers of rabbits, guinea-pigs and rats were inoculated with these animal strains and also with those derived from human cases of melioidosis.

Large doses of virulent cultures caused death from septicaemia in less than twenty-four hours. The subcutaneous inoculation of smaller amounts was always followed by a local reaction; a hard swelling formed which consisted of coagulated lymph and oedematous necrotic tissue. About the third day a small slough appeared in the middle of the swelling at the site of the

needle puncture; this gradually spread until it formed a crater-like ulcer with ragged, undermined edges and a yellowish slough in the centre. When only a very small quantity of an old laboratory culture was injected, the local lesions healed and the animal lived for two or three weeks. When it died, the lymphatics leading from the point of inoculation were sometimes enlarged and caseous, like farcy pipes, while the scapular and inguinal glands were swollen and filled with thick yellow pus from which *B. whitmori* was easily cultivated. In those animals which lived long enough the organisms were carried by the blood stream to the lungs, spleen and other viscera, where they produced the typical nodules of melioidosis.

Melioidosis, like plague, can be inoculated successfully by scarification. Merely scratching the skin with a knife, contaminated with infective material, is usually sufficient to convey the disease. The skin of the abdomen of a couple of rabbits was lightly pricked with a needle which had been dipped into a culture of *B. whitmori*. One of the animals died on the eleventh day and the other on the thirteenth. Nodules were found at the site of inoculation and in the spleen; but there was no nasal discharge in either of the cases.

All the inoculated animals died, but the respiratory system was not affected to the same degree as it was in the cases which occurred naturally, and in no instance was there any milky discharge from the eyes and nose. It was clear to us that the counterpart of the disease, as we had seen it occur spontaneously in rabbits and guinea-pigs, was not produced by inoculation of the virus into the skin; consequently we turned to investigate the possibility of the infection being conveyed to animals in their food. Fresh vegetable leaves were sprinkled with a culture of *B. whitmori* and fed to rabbits and guinea-pigs. Within two or three days the nose and eyes became moist, soon there was a milky discharge, the breathing became obstructed and the animals died in a week or ten days with all the signs and symptoms of the disease, as we had seen it occur naturally. A plug of cotton-wool moistened with an emulsion of the organism and rubbed on the nose or tongue is sufficient to cause infection.

Strauss's reaction, which is looked upon as a cardinal test for the glanders bacillus, is produced in the same manner by the bacillus of melioidosis. If a small quantity of an emulsion of either organism be inoculated into the peritoneal cavity of a male guinea-pig, within three days the testes become swollen and inflamed; subsequently they are converted with a caseous mass to which the skin is adherent. Owing to the great pathogenicity of *B. whitmori*, a smaller quantity must be injected than when *B. mallei* is employed, otherwise the animal may die in a couple of days from septicaemia.

Rats, cats, monkeys and goats are susceptible to melioidosis. We have seen several rats and one cat which died from the disease contracted under natural conditions; we have infected goats by inoculation and a monkey by mixing a culture of the organism with its food. Rats are more resistant to the disease than rabbits and guinea-pigs. Several rats (*Mus griseiventer*) were

inoculated subcutaneously, one lived for thirty-one days and another for as long as fifty days, after inoculation, before they died with caseous masses in their lungs and other viscera. There was no nasal discharge in the inoculated rats, nor was there any in the rats which became infected naturally.

In a comparative study of *B. whitmori* and *B. mallei* it is obviously of paramount importance to investigate the pathogenicity of *B. whitmori* for horses, and we are indebted to Major S. L. Symonds, B.V.Sc., for procuring animals and inoculating them for us. Five Java ponies and two Australian horses, none of which reacted to mallein, were inoculated with four strains of *B. whitmori* from human cases of melioidosis. Three of these strains had been isolated recently and were very virulent for rodents. In one instance, a thousand million organisms from a glycerine agar slope, inoculated direct with pus from an abscess in the parotid gland of a man suffering from melioidosis, were injected subcutaneously into the neck of a Java pony. Five of the animals were inoculated with saline emulsions of agar cultures and two with broth cultures. One was inoculated with the virus intravenously, one intra-nasally and the rest subcutaneously. The number of organisms injected in each case varied from about one thousand million to ten thousand million. The results were almost entirely negative; none of the horses developed a general infection. In three of them there was a moderate degree of fever which lasted for four or five days. The subcutaneous inoculations produced a localised swelling which supplicated and after discharging some thick pus left an ulcer some 5 centimetres in diameter. This lesion healed in a few weeks and the animal was none the worse.

Three of the horses were killed six months after inoculation and three died from kumrie and old age within eight weeks. No signs of melioidosis were seen in any of them, nor could *B. whitmori* be cultivated from the tissues or viscera. One of the animals remains in good health. We must therefore conclude that *B. whitmori* is not pathogenic for horses, even when it has been newly isolated from a case of melioidosis and is highly virulent for rodents.

5. THE INOCULATION OF ANIMALS WITH *B. MALLEI*.

The strains of the glanders bacillus which were in our possession had been grown for long periods on artificial media and had lost almost all their pathogenicity for animals. Two rats which we inoculated with *B. mallei*, Java, remained in good health; rats of the same species (*Mus griseiventer*) inoculated with similar amounts of laboratory cultures of *B. whitmori* died with chronic melioidosis in two or three months. The latter organism is much more virulent when it is newly isolated; two rats which were inoculated with the first subculture of the "Warton" strain of *B. whitmori*, isolated from a human liver, died from septicaemia in less than twenty-four hours.

Three guinea-pigs were inoculated subcutaneously, one with about four thousand million, and two with two thousand million, *B. mallei*, Java. Each one developed an abscess at the site of inoculation, but they all recovered

and, when they were killed a month later, no signs of disease could be found. A fourth guinea-pig inoculated subcutaneously with two hundred million organisms of the same culture showed neither local nor general signs of disease. Inoculation of a similar quantity of *B. whitmori* invariably causes death.

A rabbit which was inoculated by introducing a loopful of *B. mallei*, Java, into the anterior nares died seventy-two days later from scabies. There were no indications of glanders nor was the bacillus recovered from the organs.

A second rabbit, inoculated with two hundred million organisms of the same culture, developed a small abscess at the site of injection. It died forty-eight days later from intestinal obstruction, but there were no other lesions except the partly healed abscess, and *B. mallei* was not cultivated from the body. A guinea-pig inoculated with a culture of mixed organisms from the abscess remained healthy.

The *B. mallei* strain "Muktesar" had still less effect on the animals inoculated, for it did not produce even a localised abscess. Pledgets of cotton wool moistened with a culture were inserted into the nostrils of a guinea-pig and of a rabbit, but the animals remained in good health. Guinea-pigs and rabbits inoculated in the same way, with cultures of *B. whitmori*, develop melioidosis and die.

A rabbit and a guinea-pig were inoculated subcutaneously, the one with five hundred million organisms and the other with two hundred million, but they both remained healthy. The Muktesar strain of *B. mallei* had obviously become avirulent. A horse inoculated subcutaneously with 1.0 c.c. of a culture in blood-broth, forty-eight hours old, manifested no sign of glanders. This horse did not react to mallein prepared in the Imperial Bacteriological Laboratory at Muktesar, and when it was killed, four months later, no lesions could be found.

The Lister Institute stock strain of the glanders bacillus known as *B. mallei* A. also proved to be non-virulent. A guinea-pig inoculated subcutaneously with a hundred million organisms developed a small abscess, but it healed in a few days and when the guinea-pig was killed, four months later, there were no signs of disease.

The inoculation of a horse had a similar effect. A thousand million organisms from an agar culture, of *B. mallei* A., emulsified in salt-solution and injected beneath the skin of the neck, produced a local abscess which healed in three weeks and no lesions were found when the horse was killed four months later.

6. THE SEROLOGICAL REACTIONS OF *B. WHITMORI* AND *B. MALLEI*.

When we began to investigate the agglutination reactions of melioidosis and to compare them with those of glanders we soon found that all our cultures of *B. whitmori* were serologically identical, but the five *B. mallei* strains were sharply divided into two groups or families, one of which is

clearly related to *B. whitmori*, while the other is more distantly connected with it. This conclusion was amply confirmed by the results of absorption tests and complement fixation.

The blood of the patient, Ragaviah, who was suffering from chronic melioidosis, agglutinated the Java and Muktesar strains *B. mallei* to the full titre of 1 : 4000, and the Minett strain to 10 per cent. of that amount. The Muktesar strain of *B. mallei* absorbed, from the blood of the patient, the agglutinins for the Whitmore bacillus cultivated from his own lesions, but the Minett strain did not. These reactions emphasise the relationship of *B. whitmori* to *B. mallei*; where it differs from *B. mallei* it always differs in a positive direction except that it is not pathogenic for horses. It is more robust and powerfully active than *B. mallei*, both within the body and outside it; it causes death more quickly in man and in laboratory animals; it grows more rapidly in artificial media; it forms a corrugated growth on glycerine agar; it takes only a few days to liquefy gelatine instead of several weeks, and, greatest difference of all, it is actively motile.

(a) *Agglutination reactions.* For the purpose of investigating the relationship of these organisms, we prepared ten immune sera by inoculating a series of rabbits with five strains of *B. whitmori* and five of *B. mallei*. The five strains of *B. whitmori* comprised three of human origin, namely, *Ragaviah* (corrugated), *Mananay* and *Warton*, and also two from animal sources, *Rabbit 45* (mucoid) and *Jacques's rat*. *B. mallei* sera were prepared with the five strains in our possession, viz. *Muktesar*, *Java*, *Minett*, *Egypt* and *A*. We have indicated the source of these cultures in the preceding pages of this paper. Each of the ten serums was put up in agglutination tests with emulsions of the fourteen strains of *B. whitmori* and the five strains of *B. mallei*, of which we have already given details. The results were as follows:

(1) Serum *B. whitmori*, *Ragaviah*.

Agglutinated all the strains of *B. whitmori* to full titre.

Agglutinated *B. mallei*, *Muktesar*, and *B. mallei*, *Java*, to full titre.

Did not agglutinate the following strains of *B. mallei*, viz. *Minett*, *Egypt* and *A*.

(2) Serum *B. whitmori*, *Mananay*.

Agglutinated all strains of *B. whitmori* to full titre.

Agglutinated *B. mallei*, *Muktesar*, and *B. mallei*, *Java*, to full titre.

Agglutinated *B. mallei*, *Minett*, *B. mallei*, *Egypt*, and *B. mallei A.*, to 3 per cent. of full titre.

(3) Serum *B. whitmori*, *Warton*.

Agglutinated all the strains of *B. whitmori* to full titre, except *Ragaviah* (corrugated) and *Mananay*, which it agglutinated to 50 per cent.

Agglutinated *B. mallei*, *Muktesar*, and *B. mallei*, *Java*, to full titre.

Agglutinated *B. mallei*, *Egypt*, and *B. mallei A.*, to 10 per cent.

Did not agglutinate *B. mallei*, *Minett*.

(4) Serum *B. whitmori*, *Rabbit 45* (mucoid).

Agglutinated all strains of *B. whitmori* to full titre, except *Mananay* and *Warton*, which it agglutinated to 25 per cent.

Agglutinated *B. mallei*, *Muktesar*, to full titre, and *B. mallei*, *Java*, to 25 per cent.

Did not agglutinate *B. mallei*, *Minett*, *B. mallei*, *Egypt*, or *B. mallei A.*

(5) Serum *B. whitmori*, Jacques's rat.

Agglutinated all strains of *B. whitmori* to full titre.

Agglutinated *B. mallei*, Muktesar, to 10 per cent.

Agglutinated *B. mallei*, Java, to 25 per cent.

Agglutinated *B. mallei*, Minett, to 2 per cent.

Agglutinated *B. mallei*, Egypt, and *B. mallei* A., to 10 per cent.

(6) Serum *B. mallei*, Muktesar.

Agglutinated all strains of *B. whitmori* between 10 and 20 per cent.

Agglutinated *B. mallei*, Java, to full titre.

Agglutinated *B. mallei*, Minett, *B. mallei*, Egypt, and *B. mallei* A., to about 2 per cent.

(7) Serum *B. mallei*, Java.

Did not agglutinate *B. whitmori* strains.

Agglutinated *B. mallei*, Muktesar, to full titre.

Did not agglutinate *B. mallei*, Minett, *B. mallei*, Egypt, or *B. mallei* A.

(8), (9) and (10). Sera, *B. mallei*, Minett, *B. mallei*, Egypt, and *B. mallei* A.

Did not agglutinate *B. whitmori* strains.

Did not agglutinate *B. mallei*, Muktesar, and *B. mallei*, Java.

Agglutinated to full titre, *B. mallei*, Minett, *B. mallei*, Egypt, and *B. mallei* A.

(These three sera were of comparatively low titre, about one in two thousand.)

Judged by the results of these agglutination tests the organisms fall into three groups: the first contains all the strains of *B. whitmori*, the second consists of *B. mallei*, Muktesar and Java, the third comprises the three strains of *B. mallei* from the National Collection of Type Cultures, namely, Minett, Egypt and A.

(b) *The absorption of agglutinins.* This method was employed to test the validity of the conclusions arrived at from a study of the agglutination reactions. First the five strains of *B. whitmori* which had been used in the production of immune sera were examined, and as they proved to be serologically identical only the strain known as *Ragaviah* (corrugated) was used in these experiments. Both *B. mallei*, Muktesar, and *B. mallei*, Java, were employed, and *B. mallei*, Minett, was selected to represent the third group. The results were as follows:

(1) Serum *B. whitmori*, *Ragaviah*.

Culture *B. mallei*, Minett, did not absorb the agglutinins from this serum.

„ *B. mallei*, Muktesar, absorbed all the agglutinins.

„ *B. mallei*, Java, absorbed 50 to 75 per cent.

(2) Serum *B. mallei*, Muktesar.

Culture *B. mallei*, Minett, did not absorb the agglutinins from this serum.

„ *B. mallei*, Java, absorbed 50 per cent.

„ *B. whitmori*, *Ragaviah*, absorbed all the agglutinins.

(3) Serum *B. mallei*, Java.

Culture *B. mallei*, Minett, did not absorb the agglutinins from this serum.

„ *B. mallei*, Muktesar, absorbed all the agglutinins.

„ *B. whitmori*, *Ragaviah*, absorbed all the agglutinins.

(4) Serum *B. mallei*, Minett.

Culture *B. mallei*, Muktesar, did not absorb the agglutinins from this serum.

„ *B. mallei*, Java, did not absorb the agglutinins.

„ *B. whitmori*, *Ragaviah*, did not absorb the agglutinins.

These absorption tests show that Whitmore's organism is serologically identical with the strain of the glanders bacillus which we obtained from Muktesar. Cross absorption between them is complete.

The Java strain of *B. mallei* differs from these two, because it contains a smaller number of antigenic components. Both *B. whitmori* and *B. mallei*, Muktesar, absorbed all the agglutinins from the Java serum; but the Java bacillus did not remove more than 75 per cent. of the agglutinins from either the *B. whitmori* or the Muktesar serum.

B. mallei, Minett, is only distantly related to *B. whitmori* and to the Muktesar and Java strains of *B. mallei*. A Minett immune serum agglutinated none of them, and none of them absorbed the agglutinins from it. *B. mallei*, Minett, removed none of the agglutinins from sera prepared with the Muktesar and Java strains of *B. mallei*, or from a serum prepared with *B. whitmori*. It was not agglutinated by any of these three sera to more than 10 per cent. of their full titre. There is, however, a group relationship between this organism and the other strains which is shown by the fact that the blood of the melioidosis patient, Ragaviah, agglutinated it to 10 per cent. of the full titre and these agglutinins were removed by saturation with the patient's own bacillus.

(c) *Fixation of complement.* Acting on the advice of Sir Arnold Theiler, whom we had consulted about melioidosis, we carried out complement fixation tests with the five following strains and their corresponding immune sera: *B. whitmori*, Ragaviah, and *B. mallei*, strains Muktesar, Java, Minett and A. The outcome of these reactions emphasises the close kinship of the *B. mallei* strains Muktesar and Java, with *B. whitmori*, and differentiates them quite clearly from the Lister Institute group Minett and A. The results are as follows:

(1) Serum *B. whitmori*, Ragaviah.

With antigen *B. whitmori*, Ragaviah, fixed 8 doses of complement (or more).

„	„	<i>B. mallei</i> , Muktesar,	„	5	„	„	„
„	„	<i>B. mallei</i> , Java,	„	5	„	„	„
„	„	<i>B. mallei</i> , Minett,	„	0	„	„	„
„	„	<i>B. mallei</i> A.	„	0	„	„	„

(2) Serum *B. mallei*, Muktesar.

With antigen *B. whitmori*, Ragaviah, fixed 8 doses of complement (or more).

„	„	<i>B. mallei</i> , Muktesar,	„	8	„	„	„
„	„	<i>B. mallei</i> , Java,	„	8	„	„	„
„	„	<i>B. mallei</i> , Minett,	„	0	„	„	„
„	„	<i>B. mallei</i> A.	„	0	„	„	„

(3) Serum *B. mallei*, Java.

With antigen *B. whitmori*, Ragaviah, fixed 8 doses of complement (or more).

„	„	<i>B. mallei</i> , Muktesar,	„	8	„	„	„
„	„	<i>B. mallei</i> , Java,	„	8	„	„	„
„	„	<i>B. mallei</i> , Minett,	„	0	„	„	„
„	„	<i>B. mallei</i> A.	„	0	„	„	„

(4) Serum *B. mallei*, Minett.

With antigen <i>B. whitmori</i> , Ragaviah, fixed 0 doses of complement				
„ „ <i>B. mallei</i> , Muktesar,	„	0	„	„
„ „ <i>B. mallei</i> , Java,	„	0	„	„
„ „ <i>B. mallei</i> , Minett,	„	8	„	„ (or more)
„ „ <i>B. mallei</i> A.	„	8	„	„

(5) Serum *B. mallei* A.

With antigen <i>B. whitmori</i> , Ragaviah, fixed 0 doses of complement				
„ „ <i>B. mallei</i> , Muktesar,	„	0	„	„
„ „ <i>B. mallei</i> , Java,	„	0	„	„
„ „ <i>B. mallei</i> , Minett,	„	6	„	„
„ „ <i>B. mallei</i> A.	„	8	„	„ (or more).

The examination of the five strains of glanders bacilli from Java, Muktesar and the National Collection at the Lister Institute shows that the infecting agent in glanders is not always the same bacillus; a group of organisms, differing from each other in serological properties, has been included under the name *B. mallei*. Possibly the strains of the glanders bacillus which predominate among country-bred ponies in one part of the world, are not the same as those which are common in another, and these, again, may differ from the strains found in Arab horses.

B. whitmori differs from the strains of *B. mallei*, which we have examined, by reason of its growth on laboratory media and its active motility; but its immunity reactions show that it is closely related to certain of them and that serologically it is almost identical with the strain of *B. mallei* which we obtained from Muktesar. *B. whitmori* must, therefore, be regarded as a member of the glanders group which possesses certain peculiar physical attributes, namely, motility in young cultures and a corrugated appearance on glycerine agar.

7. SUMMARY.

(1) It is impossible to distinguish the lesions of melioidosis from those of glanders by means of inspection with the naked eye or even with the microscope.

(2) The symptoms and course of the two diseases are similar. Melioidosis generally runs a more acute course.

(3) The mallein test was applied in a case of chronic human melioidosis and gave a positive reaction. The mallein was obtained from Java.

(4) The blood of this chronic case agglutinated the Muktesar and Java strains of *B. mallei* to full titre and the Minett strain to 10 per cent. of this amount. One of these strains (Muktesar) absorbed the agglutinins for the homologous bacillus.

(5) There are very few horses in the Federated Malay States. Melioidosis is primarily a disease of rodents. All attempts to infect horses have been unsuccessful.

(6) Melioidosis broke out spontaneously among the rabbits, guinea-pigs and rats at the Institute for Medical Research in 1913. The animals became infected through eating contaminated food.

(7) Cases of infection in wild rats and in a domestic cat have occurred far away from this laboratory and independently of it.

(8) Rabbits, guinea-pigs and rats have been infected experimentally, by feeding, by subcutaneous inoculation, by scarification and by the application of infective material to the nasal mucosa.

(9) *B. whitmori*, whose growth in cultures occur in two forms, a commoner corrugated form and a mucoid form which gives origin to the corrugated type.

(10) *B. whitmori* differs from *B. mallei* in the following particulars. It is actively motile. It forms a corrugated growth on glycerine agar, a white opaque growth on ordinary agar and a pellicle on the surface of broth. It grows more rapidly than *B. mallei*, and it liquefies gelatine in a few days.

(11) *B. whitmori* resembles *B. mallei* in the following particulars. The morphology of the organisms is similar. Young cultures of the mucoid form are indistinguishable from *B. mallei*, by inspection. The growth of the mucoid form on potato is similar. The action on milk and carbohydrates differs in degree only. Both organisms produce Strauss's reaction in guinea-pigs.

(12) Five strains of *B. mallei* were compared with fourteen strains of *B. whitmori*. The cultures of *B. mallei* comprised three from the National Collection at the Lister Institute, one from Muktesar and one from Java. The cultures of *B. whitmori* had been isolated, some from human cases of melioidosis and some from animals which had acquired the disease naturally.

(13) The serological reactions of these organisms, namely agglutination, absorption and complement-fixation tests, showed that the cultures of *B. whitmori* were a homogeneous group, but the five strains of *B. mallei* were sharply divided into two sub-groups by their serological reactions. One sub-group includes the Muktesar and Java strains; the other comprises the three strains from the National Collection (Minett, Egypt and A.).

(14) The first sub-group of *B. mallei* is very closely related to *B. whitmori*. The serological reactions of the strain from Muktesar are almost identical with those of *B. whitmori*.

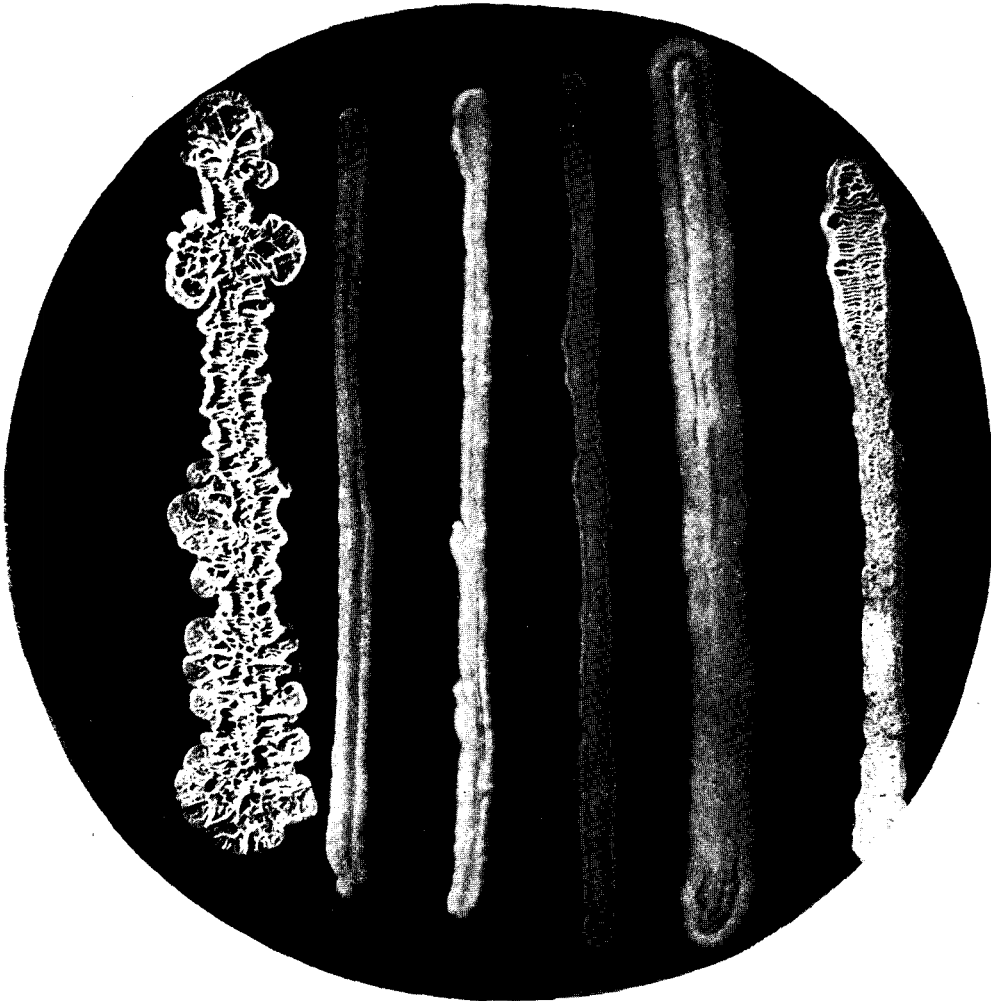
(15) The three strains of *B. mallei* from the National Collection, which form the second sub-group, are only distantly related to the Muktesar sub-group and to *B. whitmori*.

8. CONCLUSIONS.

B. whitmori is, serologically, almost identical with a strain of *B. mallei* from Muktesar, but it differs from it by reason of its motility and the character of its growth on laboratory media.

A strain of *B. mallei* obtained from Java is closely related to these organisms.

Three strains of *B. mallei*, from the National Collection of type cultures



at the Lister Institute, are distantly related to *B. whitmori* and to the strains of *B. mallei* from Muktesar and Java.

The causative organism of glanders is not always one and the same bacillus. A group of organisms differing from each other in serological properties has been included under the name *B. mallei*. *B. whitmori* is a member of this group, which has been found in rodents and in man.

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DESCRIPTION OF PLATE X.

B. whitmori and *B. mallei* on glycerine-agar. Seventy-two hours' growth.

Reading from left to right: *B. whitmori* (rat); *B. mallei* (A); *B. mallei* (Java); *B. mallei* (Muktesar); *B. whitmori* (mucoid); *B. whitmori* (cat).

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