

CHROMOBACTERIUM VIOLACEUM INFECTION IN A PIG

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SUMMARY.

A *Chromobacterium* was isolated on 10% sheep's blood agar and McConkey agar from abscesses in the lung, liver and mammary region and also from the swollen mediastinal lymph nodes of a pig. This organism grew at 25 or 37 deg. C. but not at 4 deg. C. It was haemolytic and produced a violet pigment on blood agar. Gelatin and Loefflers media were liquefied. In liquid media a pale violet pellicle was formed. The organism was pathogenic for mice and mildly pathogenic for guinea pigs.

Three other strains biochemically and morphologically similar to the pig strain and with similar pathogenicity for guinea pigs and mice were recovered from muddy water.

The nomenclature of this species is discussed. Our strains appear to fit most closely with *Chr. violaceum* var. *manilae*.

I. INTRODUCTION.

It is generally believed that the chromobacteria are not pathogenic. Wilson and Miles (1954) did not record any pathogens amongst the species described by them. However, Woolley (1904) isolated a strain causing death of carabao in Manila, and in Louisiana, Schattenberg and Harris (1942) recorded "a definite and rapidly fatal infection of the human subject with *Chromobacterium violaceum* var. *manilae*." Sneath, Whelan, Bhagwan Singh and Edwards (1953) described a fatal pyaemia in a soldier caused by a similar organism. These authors stated that from 1904 until 1952, 13 cases were recorded in man. Of these, eight died, two recovered and in three the outcome was not stated.

Seppel, Medina and Atwood (1954) isolated a strain from pigs in Georgia and showed their strain to be pathogenic for guinea pigs, mice and pigs. The strain isolated by us was from extensive lesions in the viscera of a pig. Other strains isolated from muddy water were pathogenic for mice and guinea pigs.

II. EXAMINATION OF SPECIMEN.

The viscera and mammary tissue from a young sow killed at a Townsville slaughterhouse were submitted to the laboratory by Mr. K. C. Beaumont for examination. The mediastinal lymph nodes were enlarged. There were numerous small abscesses about 3 mm. in diameter in the lungs, which also contained a few larger abscesses. Abscesses up to 2 cm. across were distributed throughout the liver. The larger liver and lung lesions had firm, light-cream, caseous centres. The border of this caseous material was conspicuously crenated. In the mammary tissue the lesions consisted of abscesses containing semi-fluid pus.

Microscopically, the lesions were characterised by a subacute inflammatory response. In the sections from affected tissue round cells were the dominating infiltrating elements. Macrophages and plasma cells were evident. In only limited areas was there any gross accumulation of polymorphs. As is common in abscesses in pig tissues, eosinophils were evident.

A gram-negative bacillus was isolated on 10% sheep blood agar and McConkey agar. Strains were obtained from mammary tissue lesions, lung abscesses, mediastinal lymph node and liver abscess.

III. DESCRIPTION OF THE ORGANISM.

Growth Characteristics.—After 24 hours at 37 deg. C. in an aerobic atmosphere, the colonies on 10% sheep blood agar were purple, hemispherical, smooth with a glistening surface and about 1 mm. in diameter. There was haemolysis around the colonies. The colony form was similar on McConkey agar, 5% glycerol agar and nutrient agar. On further incubation for several days, the colony form was the same but colony size increased to about 4 mm. diameter. There was no growth on "Difco" S.S. agar. Nutrient broth after 24 hours' growth showed turbidity with the formation of a purple surface pellicle. On further incubation, the turbidity increased and after a few days a fine white powdery sediment formed at the bottom of the medium. The purple pigment was soluble in alcohol but insoluble in water and chloroform.

Morphology.—The organism was a gram negative, non acid-fast bacillus, approximately 0.5μ wide and $1-2\mu$ long. In young cultures some organisms showed bipolarity. No spores or capsules were formed. It was motile usually with a single polar flagellum and occurred singly or in short chains.

Atmospheric Requirements.—Good growth occurred on 10% sheep's blood agar, aerobically and anaerobically. No pigment was produced anaerobically but it rapidly developed when the cultures were exposed to air.

Temperature Requirements.—On 10% sheep's blood agar growth was good at 25 deg. C., 30 deg. C., and 37 deg. C. During the first 24 hours' growth, colony formation was noticeably larger at 37 deg. C. There was no growth at 4 deg. C.

Biochemical Reactions.—In the carbohydrates routinely used in our diagnostic work, acid but no gas was produced from:—

Glucose after 24 hours at 37° C.

Sucrose after 48 hours.

Lactose and mannite after several days.

Maltose was not fermented.

The organism reduced methylene blue and nitrate and produced hydrogen sulphide, ammonia and catalase. It liquefied Loeffler's medium and gelatin.

Methyl red, Voges-Proskauer, indole and urease tests were negative.

Litmus milk was acidified and clotted slowly with slow digestion of the clot.

Sensitivity Tests.—Sensitivity tests with Evans "Sentest" tablets on 10% sheep blood agar as basal medium showed the organism to be resistant to penicillin and erythromycin, partially resistant to streptomycin and aureomycin, but sensitive to chloramphenicol, oxytetracycline and tetracycline.

IV. PATHOGENICITY FOR LABORATORY ANIMALS.

The organism was pathogenic in large doses for guinea pigs and mice. Mice inoculated intraperitoneally with 0.1 ml. of a 24-hour broth culture died overnight. A guinea pig inoculated intraperitoneally with 0.25 ml. of the same culture died in 36 hours. The organism in each case was recovered from the liver, lung and spleen of the experimental animal.

In smaller doses mice were susceptible but guinea pigs somewhat resistant.

A mouse inoculated intraperitoneally with 0.02 ml. of a 1/20 dilution (0.001 ml.) of a 24-hour broth culture died overnight. Necropsy showed a focal necrosis of the liver and the organism was isolated from liver, lung and spleen. A guinea pig was inoculated intraperitoneally with 0.1 ml. of a 1/20 dilution (0.005 ml.) of the same culture. There was no evidence of infection in this animal when it was killed fourteen days later. Three other guinea pigs inoculated intraperitoneally with 0.3 ml. of a 1/20 dilution (0.015 ml.) 0.5 ml. of a 1/20 dilution (0.025 ml.) and 0.5 ml. of a 1/10 dilution (0.05 ml.) of a similar 24-hour broth culture were not clinically affected. Fourteen days after inoculation they were killed. Four to six small abscesses of about 1 mm. diameter were found in the liver of each animal. The organism was cultured from these sites in which it had apparently localised.

V. OCCURRENCE OF THE ORGANISM.

Water and soil from a swamp on the Animal Health Station were examined. This swamp is eight miles from the piggery from which the infected animal came and it is on a different water shed.

The muddy water was centrifuged and a sample from the surface of the deposit was sown on 5% glycerol agar and McConkey agar.

Eight samples of water containing varying amounts of soil were examined from this particular swamp and three strains of chromobacteria were isolated.

The three strains isolated from the soil and water (Strains 2, 3, 4) were morphologically similar to the organism isolated from the pig described above (Strain 1). The only variations from the description given for Strain 1 were:—

Strain 2: Acid but no gas was produced in sucrose after 24 hours, weak acid but no gas in maltose after seven days. It gave a weak positive methyl red test.

Strain 3: Acid but no gas was produced in sucrose after 24 hours, weak acid but no gas in maltose after seven days.

Strain 4: Sucrose was not fermented. Weak acid but no gas was produced in maltose after seven days. It gave a weak positive methyl red test. Methylene blue was not reduced.

Pathogenicity tests with mice and guinea pigs gave similar results to Strain 1 but Strain 3 killed a guinea pig overnight when 0.3 ml. of a 1/20 dilution (0.015 ml.) of a 24-hour broth culture was given intraperitoneally.

VI. DISCUSSION.

Sneath (1956) has pointed out the confusion that exists in the classification of chromobacteria. This confusion and the general conception that *Chromobacterium violaceum* is non-pathogenic may have caused some pathogenic chromobacteria to be overlooked. As good growth occurs at 37° C. the organism isolated by us does not fit into Bergey's classification of the chromobacteria. However, it resembles the bacterium described by Woolley (1904), Schattenberg and Harris (1942) and Sneath *et al.* (1953). These articles leave little doubt that *Chr. violaceum* var. *manilae* is pathogenic.

The strain described by us almost certainly belongs to the mesophil group listed by Sneath (1956) as *Bacillus violaceus manilae* Woolley; *Chromobacterium violaceum manilae* Ford; *Chromobacterium violaceum* var. *manilae* Schattenberg and Harris.

It differs from Woolley's organism in that it is a facultative anaerobe while his is recorded as obligate aerobe. The pigment is also said to be slightly soluble in water. In our specimen, mixing of culture and water gave this impression but when the mixture was centrifuged the supernatant remained clear.

Bergey (1948) says that *Chromobacterium ianthinum* may be the same species as *Chr. violaceum manilae*, Ford but *Chr. ianthinum* is described as a bacillus 1.5 μ to 5.0 μ long with peritrichous flagella. *Chr. violaceum manilae* is a smaller organism with a single flagellum.

The organism described by Schattenberg and Harris (1942) was similar to ours in pathogenicity for mice and guinea pigs. Also, Schattenberg and Harris (1942) described the abscesses in their human case as bizarre-shaped pulmonary and hepatic nodules. In the lesions described by us in the pig irregular-shaped abscesses were a striking feature of the post-mortem examination.

From our observations the organism would appear to be common in the Townsville area but no other cases of infection have become known to us. Therefore, it seems that even though it can cause extensive lesions, the organism only rarely affects animals.

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