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Experimental Studies upon *B. violaceus*, Isolated from a Fulminating Fatal Human Infection.*

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Bacillus violaceus (*Chromobacterium violaceum*) is considered a common saprophytic microorganism occurring in soil, water, sewage and contaminated food. It is generally regarded as non-pathogenic for man and lower animals.

Woolley¹ reported fatal infections of caribao in Manila with this microorganism which resembled hemorrhagic septicemia of cattle. He also established the pathogenicity of the isolated culture but did not find evidence of toxin-production. Recently Black and Shahan² and Soule³ reported instances of human infection with *B. violaceus* which persisted for many months and finally proved fatal. Soule demonstrated pathogenicity for laboratory animals.

The culture employed in the present experiments was isolated in pure state on ordinary media from the lesions present in various organs at the necropsy of a human case. The infection arose from a wound of the foot. There ensued a marked inguinal adenitis, septicemia, as shown by a positive blood culture, and death within a week.

Studies of pathogenicity and animal protection have been performed with this culture.

Pathogenicity Experiments. 12 rabbits, 10 guinea pigs and 12 white mice were employed. Six rabbits received intravenously 1 cc of a saline suspension containing approximately one billion microorganisms per cc. The suspension was made from the growth of *B. violaceus* on nutrient agar. All 6 animals died in approximately 24 hours. The necropsies showed no macroscopical lesions. Cultures of *B. violaceus* were recovered from the lungs, liver, spleen, and heart's blood. The microscopical study of sections revealed degenerative changes, particularly in the liver.

Six rabbits were injected intraabdominally with a broth culture of

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¹ Woolley, P. G., *Johns Hopkins Bull.*, 1905, **16**, 89.

² Black, M. E., and Shahan, J., *J. A. M. A.*, 1938, **110**, 1270.

³ Soule, M. H., *Abs. Scientific Proceedings, Am. Assn. of Pathologists and Bacteriologists Am. J. Path.* 1939 **15** 509

B. violaceus in amounts of .5 cc, 0.75 cc, 1 cc, 1.25 cc, 1.5 cc, and 2 cc. Those receiving 2 cc and 1.5 cc died in 12 hours and 24 hours respectively. *B. violaceus* was grown from heart's blood and visceral organs. The animals injected with 1.25 cc and 1 cc died in 5 days. The lungs, spleen, and liver presented light yellow foci of necrosis varying from pin-point size up to 4 mm. Microscopically the lesions presented necrosis, karyorrhexis and karyolysis with little inflammatory cellular response. These lesions conformed in gross and microscopical appearance to those present in the human case. No violet color was present but *B. violaceus* was readily recovered in pure state from all lesions and also from heart's blood.

Ten guinea pigs were injected intraabdominally, 5 receiving 0.25, 0.5, 0.75, 1.0, and 1.5 cc of saline suspension of *Bacillus violaceus* from agar slants and contained approximately one billion bacilli per cc. Five other animals received similar injections of broth culture grown for 4 days and diluted to contain approximately one billion bacilli per cc; all died within 16 hours. No discernible lesions were found although *B. violaceus* was recovered from visceral organs and heart's blood. Microscopically, degenerative changes occurred in most parenchymatous structures. Three guinea pigs receiving 0.75, 1.0, and 1.5 cc of the saline suspension died in 24 hours and presented a few minute scattered lesions in the liver. The lungs were greatly congested. Microscopically the hepatic lesions were of a granular, necrotic type showing fragmented nuclei. *B. violaceus* was recovered from the organs and heart's blood. The 2 remaining guinea pigs receiving 0.25 and 0.5 cc have survived for several weeks.

Twelve mice were injected intraabdominally, 6 with 0.1 cc and 6 with 0.5 cc of suspensions of *B. violaceus* like those employed for the guinea pigs and died within 48 hours. Those receiving the broth culture died more quickly than those injected with the saline suspensions from agar slants. No gross lesions were produced but the microscopical study revealed evidence of toxic degenerative changes particularly in the liver lobules. *B. violaceus* grew readily from the heart's blood, liver and spleen.

To ascertain if this human strain of *B. violaceus* possessed an exotoxin, cultures were grown in flasks of broth. After incubation for 24 and 96 hours, the cultures were filtered through a Seitz filter at these respective periods. It was found that intraabdominal injections of as much as 2 cc of this sterile filtrate into white mice produced no ill effects. When these animals were later injected with fatal doses of *B. violaceus*, they remained normal although control animals

receiving the same dosage died overnight. Further tests for such protection were, therefore, made.

Animal Protection Experiments. Eight white mice were injected intraabdominally with 0.5 cc of sterile broth Seitz filtrate obtained from a 24-hour culture. After 3 days, 1.5 cc of a similar filtrate from a broth culture grown for 96 hours, was likewise administered to these animals. Two days later all animals received intraabdominally 0.1 cc of a virulent *B. violaceus* broth culture. All animals survived and showed no evidence of illness during several weeks of observation.

Of 10 control mice, 5 received intraabdominally 0.5 cc and 5 received 1.0 cc of the same culture as used for the above animals and all died overnight.

Two control mice were injected with plain sterile broth in similar amounts, route and time periods as those receiving the broth culture filtrate. The injection 48 hours later of 0.1 cc of the living culture produced death within 24 hours.

It is concluded from these observations that this strain of *B. violaceus* isolated from an unique clinical case, is pathogenic for various laboratory animals. When the inoculum permits of survival for several days, the lesions produced are similar in the gross and microscopic aspect to those of the human.

It is further shown that the injection of *B. violaceus* broth culture filtrates into white mice affords protection to doses of the living microorganism that kill control animals overnight.

Preliminary experiments with the serum of mice injected with sterile filtrates suggest that antitoxic factors are developed by which passive protection may be transferred to normal animals.

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A New Method for Regulated Vascular Obstruction.

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Restriction of blood flow in isolated vascular territories in living and unanesthetized animals has recently attracted attention. Some workers have fitted the blood vessels which are to be constricted into a type of rigid frame which reduces the diameter of the vessels.