

Experimentally Produced Wounds as a Route of Fatal Infection Caused by *Bacillus violaceus*.*

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by C. W. Duval.)

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Although *B. violaceus* is generally considered as of saprophytic and non-pathogenic nature, it is evident that at least certain strains of this microorganism demonstrate virulent and fatal infection for man and lower animals. Woolley¹ demonstrated such infection with rapid production of death in carabao or water-oxen. Black and Shahan² and Soule³ reported protracted infections in the human subject which eventually proved fatal. In the present human case to be reported⁴ infection, septicemia, and death within a week were noted in a robust adult who previously had enjoyed excellent health.

In a review of the aggregate of the instances wherein fatal *B. violaceus* infection had occurred there is reason to believe that the microorganism had gained entrance through injury or wounds of the extremities. In the case herein concerned, there was a definite history of infection through a splinter wound of the foot with pain of this extremity and accompanying adenitis. While consistent pathogenicity for lower animals has been reported for the culture isolated,⁵ the microorganism was injected by use of a syringe through the subcutaneous, intraabdominal or intravenous route.

The present experiments were undertaken to ascertain if infection with *B. violaceus* could occur through surface injury. Varied types of wounds were produced in the feet of white mice and rabbits with subsequent exposure to infection by this microorganism, thereby mimicing to a reasonable degree the condition considered as the natural atrium of bodily invasion.

White Mice. The soles of the hind feet of 10 such animals were scarified to a depth well beneath the skin by use of a scalpel. Subsequently, the wounded parts were either dipped into or swabbed with

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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 Proc. Am. Assn. Pathologists and Bacteriologists, Am. J. Path.*, 1939, **15**, 592.

⁴ In press.

⁵ Schattenberg, H. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 829.

a dilution of broth culture of *B. violaceus* contained in Petri dishes. This exposure to infection lasted for approximately a minute and was repeated 24 hours later, although the wounds at that time presented "scabbing" or crusted coagulum. All of these animals showed tumefaction and impaired function within 24 hours in the leg wherein the sole was traumatized. Within 4 to 6 days, 8 of the infected animals were very perceptibly sick. As death was impending, blood cultures were procured from the heart in 4 of the animals and all yielded heavy growths of *B. violaceus*. These animals died from the heart puncture while the 4 remaining mice survived for an additional 24 to 48 hours. Necropsies of these animals showed inguinal adenitis of varying degrees apparently contingent upon host resistance or the number of invading microorganisms. In other words, those animals surviving for the longer periods revealed a more marked adenitis. The glands were congested and swollen and in 2 instances, small areas of necrosis were noted. In the abdominal cavity, the retroperitoneal nodes presented similarly varying degrees of involvement. Multiple very minute light yellow lesions were found especially in the liver and at times in the spleen and lungs. The microscopic study of the involved structures presented necrosis of the parenchymatous cells with, at times, peripheral cellular response of the lymphoid, plasma and neutrophilic varieties. Cultures of the gross lesions and heart's blood yielded heavy growths of *B. violaceus*.

Controls. Ten white mice having normal or unabraded feet were submitted to similar contact with the *B. violaceus* suspension. These animals remained normal and unaffected.

Rabbits. Twelve such animals were employed. In 5 of these, the fur was clipped on one hind foot and a small incision was made through the skin and into the subcutaneous structure. To simulate an infected splinter, a portion of a wooden tooth-pick 6 mm in length, which had been dipped in a broth culture of *B. violaceus*, was introduced into the tissue at the upper end of the wound, parallel to the line of incision. For 3 other rabbits, after clipping the fur on the sole of one rear paw, one incision approximately 1 cm in length with 2 to 3 smaller cross incisions were made, all of which extended into the subcutaneous tissue. These incised areas together with the paws of 4 others wherein no incision was made were swabbed with a broth culture of *B. violaceus* for approximately a minute on 2 successive days.

The 5 rabbits having infected "splinters" in the feet showed within 24 hours swelling and dysfunction of the corresponding leg.

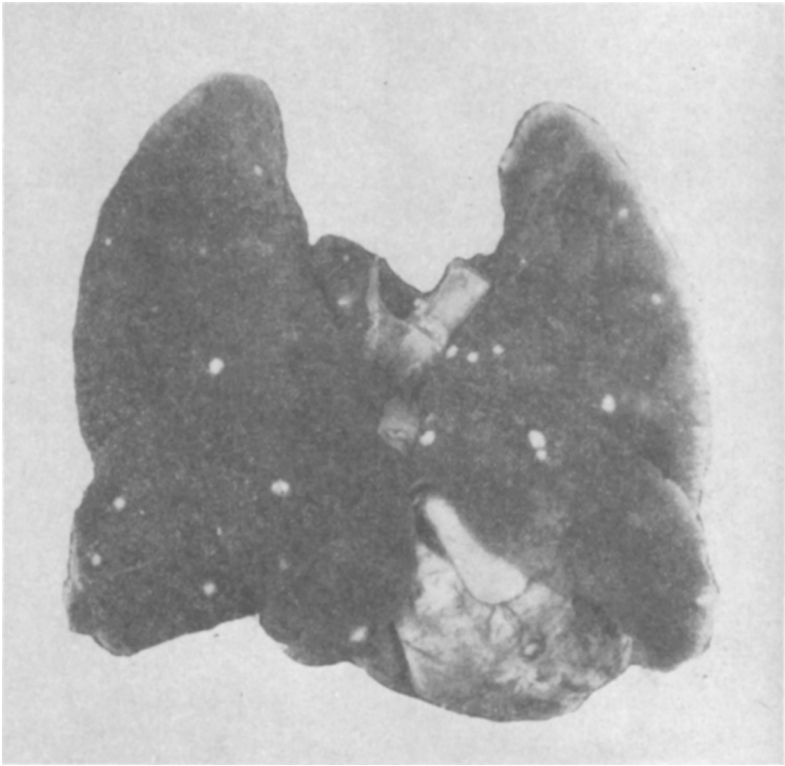


FIG. 1.

The lungs show numerous necrotic nodules of varying size and shape scattered beneath the pleural surfaces. A similar lesion is noted on the anterior surface of the heart, shown below.

They became perceptibly and progressively ill. One of these died within 4 days. At necropsy the leg was swollen but the inguinal nodes showed nothing noteworthy. The lungs contained scattered small yellow lesions measuring approximately 1 mm in diameter (Fig. 1.) A few such lesions were noted in the liver and heart wall. Microscopic study revealed similar findings as those described for the mice but to a more exaggerated extent especially in the liver. (Fig. 2.) In the lungs, marked congestion, oedema and mild pneumonitis occasionally surrounded the necrotic foci. Cultures made from the heart's blood and the lesions of the lungs, liver and feet yielded growths of *B. violaceus*. Of the 4 remaining rabbits with "splinters" one died in 8 and one in 9 days, the 2 others have survived. The necropsies of these animals showed in general the same aspects as that described for the above rabbit. There was, however, marked inguinal adenitis with some necrosis of structure.

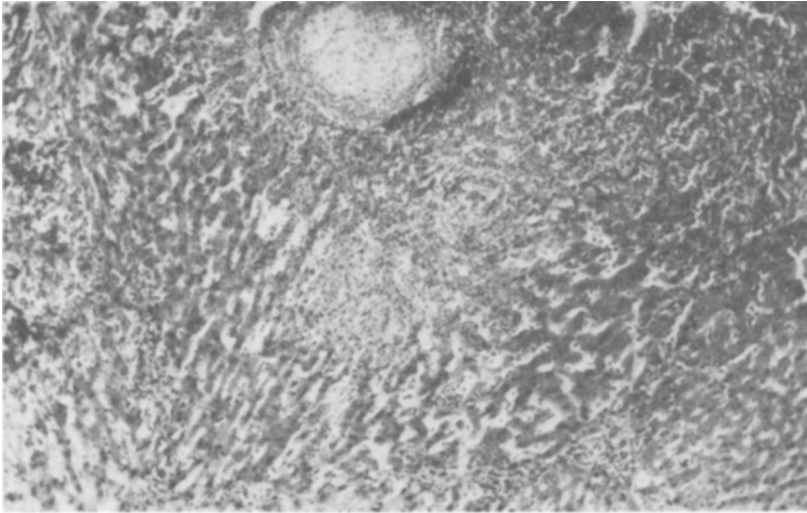


FIG. 2.

In this section of the liver there is seen a necrotized oval nodule. Below this is shown an earlier lesion of focal necrosis in which pyknosis and karyorrhexis of the nuclei has occurred.

Of the 3 rabbits wherein the crucial incisions were submitted to *B. violaceus* infection, one died in 11 days and one in 2 weeks. The remaining animal has survived. Necropsy of the 2 animals showed scattered small gross lesions in the lungs and liver. In one animal a marked inguinal adenitis was present with congestion and areas of necrosis. One of the glands was enlarged to approximately 1 cm in the longest diameter. The microscopic study was similar to that already described. Cultures of the lesions and heart's blood yielded growths of *B. violaceus*.

The 4 rabbits wherein no injury was present in the feet, although submitted to similar bacterial exposure as those with incisions, remained unaffected and normal.

Route of Infection. In these experimental injuries or wounds the invasion of *B. violaceus* must occur, through the lymphogenous or hematogenous routes or both. The marked adenitis on the one hand indicates a lymph stream invasion finally traveling to the blood circulation as shown by positive blood cultures. On the other hand the lack of notable lymph gland involvement and the accentuated extent of lesions in the lung in certain of the animals, suggest direct primary blood circulatory ingress as the principal route of infection. In either event, the likelihood and rapidity of invasion through such routes by this microorganism is clearly demonstrated.

Summary. It is shown herein that infection with *B. violaceous* may readily occur through various types of surface injury of the feet of animals. It is suggested, therefore, that ingress through external injury forms the usual portal of entry of *B. violaceous* in the natural infection. These infections are fulminating and usually fatal in a short period of time. In this connection, it appears probable that both the lymph and blood streams may form the routes of host invasion although the lymphatic pathway is emphasized. The lesions produced are similar to those found in the natural infection of carabao as well as those observed in the human infection. Although *B. violaceous* has in general been regarded as innocuous, it is further stressed herein that certain strains are definitely pathogenic and present evidence of distinct virulence. Furthermore, a likely portal of entry of the natural infection is indicated.

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Nutrition of *Tetrahymena geleii* (Protozoa, Ciliata).

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All attempts to grow *Tetrahymena geleii* in a medium of known composition have, so far, failed. Failure to obtain growth in anything but peptones led Lwoff¹ to formulate the hypothesis that the ciliate requires polypeptides and cannot utilize free amino acids. Earlier² he had hinted that the failure of growth in a mixture of silk peptone, fibrin peptone and gelatin, which contains all the known amino acids, is the result of the lack of some supplementary substance. Gelatin³ will support growth if supplemented with a small amount of yeast extract; indeed, it is stated⁴ that transplantable growth is obtained in gelatin plus thiamin and riboflavin. Silk peptone gives slight, but transplantable, growth if thiamin is added.⁵

* Aided by a grant from the Manufacturers' Research Fund for Bacteriology and Protozoology at Brown University.

¹ Lwoff, A., *Monogr. de l'Inst. Pasteur*, 1932.

² Lwoff, A., *C. R. Soc. Biol.*, 1924, **91**, 344.

³ Hall, R. P., and Elliott, A. M., *Arch. f. Protistenk.*, 1935, **85**, 443.

⁴ Hall, R. P., *Anat. Rec.*, 1940, **78** (suppl.), 164.

⁵ Lwoff, A., and Lwoff, M., *C. R. Soc. Biol.*, 1937, **126**, 644; D. M. Lilly, personal communication.