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The isolation of the B. histolyticus from the ileo-caecal region of two human intestines.

By JOHN C. TORREY.

[*From the Department of Hygiene, Cornell University Medical College, New York City.*]

The *B. histolyticus,* a member of the gram-positive, spore bearing group of anaerobes, is a strongly proteolytic and markedly pathogenic organism, which was first discovered and described by Weinberg and Sequin[1] in the course of their study of war wound infections. They isolated it from eight cases of gas gangrene, and demonstrated its remarkable proteolytic action on living muscular tissue. It was assumed that it found its way into wounds through fecal polluted soil, as was known to be the case as regards *B. tetani, B. welchii* and other typically intestinal organisms. This theory received substantiation through the report of Hall[2] of the presence of this organism in the stool of a normal adult and its isolation from arable soil by Petersen and Hall.[3]

During the course of an investigation of intestinal bacteriology and absorption of intestinal bacterial products which is being carried on in this laboratory, we have had the opportunity to study the types of bacteria present at various levels of human large intestines removed at coleoctomy operations. I am indebted to Dr. John W. Draper for this privilege, and to my associate, Dr. John W. Churchman, for obtaining the specimens at the time of operation.

Of ten intestines thus far examined, two have yielded this *B. histolyticus,* and both at the same levels of the intestine. One of these cases was a severe epilepsy in a boy (H) which had been present in an aggravated form since early childhood; the other (C) was a case of chronic and obstinate constipation with a condition of marked stasis in the caecum and colon.

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[1] Weinberg and Sequin, *La Gangrene Gazeuse,* Masson et Cie, Paris, 1917.

[2] Hall, Ivan C., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 198.

[3] Petersen, E., and Hall, I. C., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 502.

Cultural studies of the material at various segments in the intestine, which were separated by ligatures at the time of operation, showed in both instances the presence of *B. histolyticus* in predominant numbers, as far as the spore bearing anaerobes were concerned, in the lower ileum and caecum. In the lower ileum it far outnumbered other spore bearers, and in the caecum seemed to occur in numbers exceeding 1000 per mg. of the material. Lower down in the large intestine it apparently did not thrive so well; in the colon there was evidence of its presence but it was considerably outnumbered by *B. welchii* and other spore bearing anaerobes, and in the sigmoid region no evidence of its presence was observed. The material from the latter region, however, consisted only of scrappings from the intestinal wall.

The strains of *B. histolyticus* isolated from these cases were submitted to careful cultural and pathogenic tests, and were found entirely typical. They exhibited a strongly proteolytic action on milk casein, gelatin and cooked minced meat medium, producing in the latter the characteristic white balls of tyrosin crystals. In all tests these strains showed this typical peculiarity, namely, proteolysis without foul odors and with little or no gas production. None of the carbohydrates tested, glucose, lactose, saccharose, salicin, xylose, or insulin, were fermented.

The type of lesion which follows the injection of *B. histolyticus* into muscular tissue of experimental animals is unique and in itself of great differential value, with a fairly virulent strain 0.5 cc., of a 24 hour broth culture inoculated into a thigh muscle of a guinea pig will cause in 24 to 48 hours extensive digestion of the muscular tissue with marked hemorrhage but without gas formation. Comparative virulence tests with these intestinal strains, isolated from the ileums of these two cases, disclosed a marked difference in virulence. Inoculations, of graded amounts of 24-hour casein digest broth cultures, were made into the adductor muscles of large guinea pigs. It was found that 0.1 cc. of the H strain was sufficient to cause typical lesions, whereas 1.0 cc. of the C strain was required to produce a similar effect.

The fact that, except for the finding by Hall of this organism in a stool specimen, there has been no previous report of its isolation from the human intestine, would seem to indicate that it is very rarely present in this locality. M. C. Kahn[4] has recently

[4] Kahn, M. C., *J. of Infect. Diseases*, 1924, xxv, 423.

completed in this laboratory a study of the spore bearing anaerobes present in stool specimens from 72 individuals, 60 of whom exhibited symptoms more or less definitely related to the intestine, but did not find the *B. histolyticus* in any instance. I believe, however, that it occurs in the human intestine more commonly than these results would seem to indicate, and that failure to find it in stool specimens may be due to overgrowth with other organisms of the same group, such as *B. sporogenes* or *B. welchii.*

The question of the possible harmful effect of this toxin-producing organism, when vegetating in the intestine, will be discussed in subsequent communications.

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Changes in virulence and growth characteristics of bacterium lepisepticum following alterations in oxygen tension.

By LESLIE T. WEBSTER.

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

The micro-organism of the pasteurella group associated with rabbit snuffles and its complicating pneumonias, septicemias, etc., is known as *Bacterium lepisepticum.* Quite recently, De Kruif noted that a mutation of freshly isolated strains of this microbe, which took place in extract broth cultures, was favored by high concentrations of peptone, and was inhibited by undiluted serum or beef infusion. The recently isolated virulent strain which he designated as Type D was found by him to grow diffusely in serum and plain broth, to form rather opaque, fluorescing colonies on serum agar, and to have an acid agglutination zone of pH 3 to 3.5; while the mutant, which he called Type G, exhibited granular growth in fluid media, appeared as translucent, non-fluorescing colonies on serum agar, showed an acid agglutination optimum between 3.5 and 5, and was of low virulence.

This apparent physical change in the organism, together with the general distinguishing characteristics of each type, has been