

Bronfenbrenner⁴ stated that the diffusion of bacteriophage occurred even at 4°C. This diffusion factor in itself seems to us inadequate to account for the occurrence of rings of lysis. The following theory is presented as a possible explanation. The central plaque ceases to increase in size after 24 hours because by then, some inhibitory product resulting from bacterial lysis might have been accumulated. If one may be permitted to assume that this inhibitory product cannot diffuse in the agar as rapidly as the bacteriophage, then one might expect a concentric ring of partially lysed bacteria immediately surrounding the central clear plaque, to be surrounded by another zone of clearing. Thus alternate zones of clear and cloudy rings corresponding to presence and absence of bacteriolysis can be seen. The increased density of bacterial growth occurring at the outermost ring might be due to the stimulating action of the bacteriophage.

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Resistance In Vitro of *Leishmania Donovanii* to Contamination with Bacteria.

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Leishmania donovani, the causative agent of Kala-azar, is generally believed to be a delicate organism which cannot survive in the presence of common bacteria. The studies of Spagnolio¹ and those of Giugni and Benoni² tended to indicate that contamination with various common bacteria is detrimental to the culture of this parasite. The recent demonstration by Forkner and Zia^{3, 4} of viable and infective *Leishmania donovani* in the midst of numerous bacteria in the oral and nasal secretions of 13 out of 14 patients suffering from Kala-azar has, however, led us to alter our concept con-

⁴ Bronfenbrenner, J., "The Newer Knowledge of Bacteriology and Immunology," The University of Chicago Press, Chicago, 1928, p. 526.

¹ Spagnolio, G., *Malaria e Malat. d. Paesi Caldi*, 1912, **3**, 151. *Abs. Trop. Dis. Bull.*, 1912, **1**, 8.

² Giugni, F., and Benoni, F., *Malaria e Malat. d. Paesi Caldi*, 1915, **6**, 89. *Abs. Trop. Dis. Bull.*, 1915, **6**, 220.

³ Forkner, C. E., and Zia, L. S., *J. Exp. Med.*, 1934, **59**, 491.

⁴ Forkner, C. E., and Zia, L. S., *J. Exp. Med.*, 1935, in press.

cerning the resistance of this parasite to bacterial contamination and prompted us to test more specifically the exact relationship which exists *in vitro* between *Leishmania donovani* and some of the common bacteria with which they may be associated in the mammalian host.

The well-known and commonly employed N.N.N. (Novy-Mac Neal-Nicolle) medium is obviously unsuitable for our experiment, since the parasite will not grow or multiply in this medium in the presence of bacterial contamination. We found that a simple medium, with which Carter⁵ successfully cultured *Leishmania tropica* from contaminated oriental sores, was suitable for our purpose. The medium used by us was prepared with slight modification and simplification, as will be reported in our final publication.

The *Leishmania donovani* used in the experiments were obtained from 2 sources. The flagellates were obtained from a 2 or 3 weeks old culture on N.N.N. medium. Leishman-Donovan bodies were obtained from the spleens of infected Chinese hamsters. The animals were killed with ether anesthesia and the spleens removed with aseptic technique. Emulsions were made by macerating approximately one-third or one-fourth of each of the spleens in 3 or 4 cc. of sterile 0.9% sodium chloride solution, and they were then ready for use.

The bacteria employed in the experiments were from stock and recently isolated cultures either in meat infusion broth or on China blue plate. They were *Staphylococcus aureus*, *Streptococcus hemolyticus*, *Pneumococcus* type undetermined, *Pneumococcus* type 1, and *B. coli*. In the case of *Staphylococcus aureus*, *Streptococcus hemolyticus* and *Pneumococcus*, 2 small platinum loopfuls of the broth cultures, and in the case of *B. coli*, 2 colonies from the China blue plate, were inoculated at about the same time as the *Leishmania donovani* into the respective tubes to get the desired contaminations. Both the inoculated and the control tubes were left in an incubator with the temperature maintained between 20° and 22°C. Examination of each tube for presence of flagellates was accomplished by removing a drop of the medium with a platinum loop. The drop was deposited on a slide, covered by a cover slip and examined under the microscope with a high dry lens.

We found that the Leishman-Donovan bodies may flagellate in Carter's medium after being associated, for at least as long as 41 hours, with the various types of bacteria except *B. coli*, and that the

⁵ Carter, M., *Brit. Med. J.*, 1909, **2**, 647.

resulting flagellates can multiply and live for from 10 to 24 days in the original tubes without subculture. The contaminating bacteria were recovered from the respective tubes at the time when the flagellate growth was most abundant. These bacteria represent fairly well the kinds of bacteria with which *Leishmania* may come into contact when discharged from the body of their host. Our results, therefore, lend additional support to the hypothesis that the infectious agent of Kala-azar may be transmitted, at least in some cases, through the agency of the secretions from the upper respiratory and upper alimentary tracts.^{3, 4} *B. coli* is the only bacterium in our group which exhibits a definite detrimental effect on the development and survival of the Leishman-Donovan bodies. Forty-one hours after inoculation, when the other tubes were showing Leishman-Donovan bodies with beginning flagellation and when in some there were already a few actively motile flagellates, there were only degenerating Leishman-Donovan bodies in the tubes contaminated with *B. coli*. It is suggested that under ordinary circumstances, the Leishman-Donovan bodies discharged from the body through the lower alimentary tract are quickly destroyed by the ever-present *B. coli*. But if the duration of the association of these 2 in the lower intestinal tract is shortened by increased peristaltic movements as in dysentery or diarrhea, or if the parasites are protected by flakes of mucus, it would seem reasonable to think that they may survive and may also serve as a source of infection.