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Origin of L Type Colonies in Bacterial Cultures.*†

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Small secondary colonies, corresponding morphologically to young colonies of the Ll strain isolated by Klieneberger from cultures of *Streptobacillus moniliformis,* have been observed occasionally in cultures of various Gram negative bacteria.[1] In all cultures in which such secondary colonies developed a large number of bacteria swelled up into large deeply stained, spherical or fusiform bodies. It was first seen in a culture of *Bacillus influenzæ* that occasionally L type of colonies developed from the large bodies. Later this process was observed repeatedly in the cultures of 2 colon bacillus strains.

After 24 hours of growth the colonies of these strains consisted mainly of large spherical bodies. In order to observe the development of these large bodies in these 2 strains different procedures proved to be helpful. The colonies of one strain were very tenacious and when an impression of the colonies was made on blood agar plates only the large bodies, with very few bacteria, were transferred. After 6 to 12 hours fine curved filaments grew from the large bodies into the agar and after 24 hours about two-thirds of them developed into tiny L type colonies. With the other strain a similar process was observed when a broth culture was transferred on blood agar plates after varying time intervals. In the transplant made after 24 hours' growth many large bodies were visible on the agar surface. Only a few bacterial colonies developed in these plates but after 6 to 12 hours the large bodies began to germinate and produced L type colonies. Transplants made from the broth culture after 48 hours, gave only abundant bacterial growth. With either strain, L type colonies developed only at the places where large bodies were present. Therefore, it is extremely unlikely that the L type colonies develop from invisible granules attached accidentally to the large bodies or from regularly shaped bacteria. In transplants made with platinum loops from agar cultures the development

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1 Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 636.

of the large bodies into the L type colonies was difficult to demonstrate because the agar was overgrown by bacteria.

Subsequent to these observations it was found that the germination of the large bodies of *Streptobacillus moniliformis* could be observed if appropriate media were employed. The best results were obtained with an alkaline meat infusion agar (pH 8.2) without the addition of blood or ascitic fluid. A freshly isolated strain of *Streptobacillus moniliformis* was used. After 24 to 36 hours' growth on ascitic fluid media the colonies of this strain consisted mainly of large spherical bodies. On the alkaline agar macroscopic growth was observed only in thickly seeded areas. This growth consisted of regular *Streptobacilli* showing little pleomorphism. The remains of the abundant transplant degenerated and could not be stained. In the thinly seeded areas bacterial growth was absent but the large bodies remained well preserved and deeply stained. From a large number of them tiny L1 colonies were seen to grow in a manner similar to that noticed in the colon bacillus cultures. No L type colonies developed in the absence of the large bodies. Temperature between 25-30°C was most favorable for the germination of the large bodies.

According to these observations there are two stages in the development of L colonies. One first notes that the bacteria swell and form large deeply stained bodies. Under appropriate cultural conditions these germinate and produce the L type colonies. In cultures of L1 strains growth may be transmitted by small filter-passing elements. The transition from regular bacilli into the L form seems to occur through the large bodies. The significance of these tiny colonies, consisting often of very small elements, developing from the bacteria in this peculiar way, is very puzzling. The L1 strains are antigenically similar to the *Streptobacillus moniliformis*[3, 4] and occasionally revert to the typical *Streptobacillus*.[2, 4] These studies leave no doubt that the L1 strain is a variant form of the *Streptobacillus* and not an extraneous element in the cultures. In the case of other bacteria we can infer only by analogy that they represent a variant form.

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2 Dienes, L., *J. Inf. Dis.*, 1939, **65**, 24.

3 Klieneberger, E., *J. Hyg.*, 1938, **38**, 458.

4 Dawson, M. H., and Hobby, G. L., *Transactions Assn. Am. Phys.*, 1939, **44**, 329; Third International Congress for Microbiology, Abstracts of Communications, New York, 1939.