

16563 P

Isolation of L Type Colonies from Typhoid Bacilli with the Aid of Penicillin.*

L. DIENES.

From the Department of Pathology and Bacteriology, Massachusetts General Hospital, and the E. W. Lovett Memorial, Harvard Medical School, Boston, Mass.

It has been briefly reported that on blood agar plates inoculated with *H. influenzae* in the area surrounding penicillin cups colonies develop which in the appearance and in the morphology of the constituting organisms are closely similar to the L₁ developing in cultures of *Streptobacillus moniliformis*.¹ After this observation had been made, many bacterial species were studied on plates containing increasing concentrations of penicillin. L type cultures were isolated without difficulty on such plates from certain strains of *Bacteroides* and from a strain of *Flavobacterium*. Some of these strains produced similar colonies immediately after isolation without penicillin, but lost this property during prolonged cultivation.² Colonies of similar type were seen on penicillin plates inoculated with various other bacteria, but up to the present time, besides the species mentioned above, they have been isolated only from *Proteus* and typhoid bacilli. In this note the isolation of L type colonies from typhoid bacilli is described.

Two strains of typhoid bacilli were studied, one recently isolated from a carrier and an old laboratory strain of unknown origin. When the usual horse blood and ascitic agar plates containing 20 units of penicillin per cc or more were inoculated with these strains, no visible growth developed. Microscopic examination of the plates dis-

closed that some of the bacilli swelled up to large round bodies, and a few L type colonies started to develop. These remained very small and disappeared during further incubation. It was learned from experience with other bacteria that the composition of the medium exerts a great influence on the growth of these colonies. Plates of various composition were tested, and a macroscopically noticeable growth developed on nutrient agar diluted with an equal amount of broth to which 10% fresh horse serum, about 1/2% laked horse blood and 25 to 5000 units of penicillin per cc had been added. Colonies sometimes developed on aerobic plates, but more regularly under anaerobic conditions. They continued to increase in size during a week of incubation. The colonies were transferred to similar plates with a block of the agar cut out of the original culture. The transfer plates were incubated anaerobically. The colonies continued to grow on the agar block and grew into the fresh medium beneath it. A good growth of tiny colonies was obtained when the agar block was pushed on the surface of the medium after a few days and the plate was further incubated. In successive transfers the colonies grew faster and to a larger size regardless of whether or not the plates contained penicillin. Growth could be obtained aerobically and on the usual horse blood agar plates, but it remained slight and the culture died out after a few transfers. The appearance of the growth on the soft horse serum plates after the strain was well adapted to artificial cultivation is shown in Photograph I. Similar growth was obtained from both typhoid strains, but that obtained from the freshly isolated strain was more abundant and could be more easily maintained in cultivation.

This growth isolated from typhoid bacilli

* The expenses of this investigation have been defrayed in part by a grant from the Commonwealth Fund.

This is publication No. 102 of the Robert W. Lovett Memorial Foundation for the study of crippling disease.

¹ Dienes, L., *Proc. Soc. Exp. Biol. and Med.*, 1947, **64**, 166.

² Dienes, L., and Smith, W. E., *J. Bacteriology*, 1944, **48**, 125.

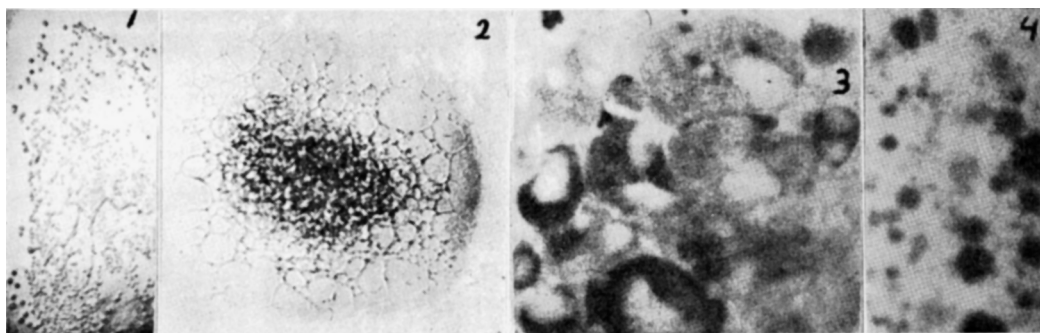


Fig. 1.

1. L type colonies from typhoid bacilli on soft horse serum plates. Slight magnification ($\times 2$). 2. One colony photographed unstained with the characteristic dense center and light periphery. ($\times 200$). 3. The periphery of a young colony consisting of vacuolated large bodies. ($\times 2000$). 4. Impression preparation from the dense center of a colony showing the small and the larger swollen forms. High magnification ($\times 3000$).

The photographs were made from cultures grown without penicillin.

differs in many respects from the usual bacilli. The young colonies consist of very small pleomorphic forms which grow into the medium and have a tendency to swell into round forms. First a dense colony embedded in the agar is formed. Later, on the surface of the colonies, the small forms swell to large round bodies and extend as a wide peripheral zone on the surface of the agar. The organisms are very soft and fragile, and the colonies often autolyze. The growth is slow and slight compared with the usual bacterial growth, and even high concentrations of penicillin exert no influence on it or on the morphology of the organisms. In all these properties the small colonies isolated from typhoid cultures are similar to the L_1 . The appearance of the colonies and the morphology of the organisms are so similar to that of the L_1 and the L type colonies isolated from *Bacteroides* that these cultures obtained from different species can hardly be distinguished on the basis of morphology alone. Their growth requirements and serological properties are different.

That the L type colonies originate from the typhoid bacilli and are not contamination in the cultures is indicated by various observations. These colonies were isolated from the cultures of 2 strains and were observed on all plates offering appropriate conditions. They developed only on plates inoculated with typhoid bacilli and not on others inoculated at the same time with various other species.

The L type colonies give a specific agglutination with typhoid sera. They originate from the bacilli in a manner similar to that of the L_1 growing from the large forms produced by penicillin. The detailed discussion of these observations must be left for future publications.

Summary. On soft horse serum agar containing high concentrations of penicillin, colonies corresponding to the L_1 of *Streptobacillus moniliformis* were isolated from typhoid bacilli. Cultures obtained from these colonies could be propagated indefinitely on similar media either with or without the addition of penicillin.