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Isolation of L Type Growth from a Strain of *Bacteroides funduliformis*.*†

LOUIS DIENES.

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

It has been described in a previous note that in cultures of certain bacterial strains belonging to various species tiny colonies, similar to young colonies of the pleuropneumonia group of organisms develop.¹ During the last year these colonies were isolated in pure culture from a strain of *flavobacterium* and one of *Bacteroides funduliformis* respectively. The colonies isolated from the *flavobacterium* always died out after 2 or 3 transplants; those isolated from the *funduliformis* apparently can be indefinitely maintained in cultivation. Colonies resembling those of the organism of pleuropneumonia bovis were first isolated by Klieneberger from cultures of *Streptobacillus moniliformis*.² She designates them with the letter L. Recently she described the isolation of similar cultures from a Gram negative streptobacillus causing suppurative lesions in guinea pigs.³ The characteristics which differentiate these L type colonies from the usual bacterial colonies are the following:

The colonies are very tiny. The growth extends not only on but beneath the surface of the medium. The growing elements of the colonies are tiny pleomorphic granules and fine filaments which swell up and may form large round bodies of 5 to 10 micra. The pleomorphism of the cultures is accentuated by the softness of the elements. They are disfigured by the slightest tearing and for this reason are not recognizable in dry smears. The colonies have a marked tendency to autolyze.

A strain of *funduliformis* was isolated from a case of generalized peritonitis. Immediately after isolation the bacterium was similar in many respects to *Streptobacillus moniliformis*. It grew in the form of Gram negative segmented filaments which in fully developed

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

² Klieneberger, E., J. Path. and Bact., 1935, **40**, 93; 1936, **42**, 587.

³ Klieneberger, E., J. Hygiene, 1940, **40**, 204.

colonies became pleomorphic and many of the bacteria swelled up into large round bodies. The colonies autolyzed within a short time and after 3 days' incubation could not be further transplanted. The strain is strictly anaerobic and not pathogenic for mice or guinea pigs. The colonies grew to a larger size and the bacterial filaments were thicker than those of *Streptobacillus moniliformis*. L type growth never developed beneath the colonies.

The growth of this strain was most abundant on rabbit serum agar plates. When fresh growth from such plates was transferred to horse blood agar plates only a few bacterial colonies developed on the thickly inoculated places. After 2 days' incubation many very tiny colonies appeared between the bacterium colonies and on the less thickly inoculated parts of the plate. These tiny colonies in a stained agar preparation were indistinguishable in appearance from young colonies of the Ll of Klieneberger. They consisted of a few deeply stained large bodies lying on the surface of agar and of small pleomorphic deeply stained granules growing like roots in several directions into the agar. The technic used for the staining of the cultures has been described previously.⁴

Squares of agar containing the tiny colonies were cut out and transferred to boiled blood ascitic fluid or rabbit serum agar plates so that the colonies were lying on the fresh medium. No bacterial growth developed in the transplants but the original L type of colonies increased considerably in size and a similar growth developed beneath the colonies in the fresh medium. Proceeding in a similar way, in one series a good growth of L type of colonies was obtained in 12 consecutive transfers. If an agar block with well developed colonies was pushed over the surface of the medium a few colonies developed on the smeared area but thus far the cultures can be kept growing in successive transfers only beneath the agar blocks. The fully developed colonies as well as the young ones were similar to the Ll of Klieneberger. They consisted of similar elements and their surface was covered, as in the case of Ll colonies, with deeply stained large round bodies. It was noticeable, however, that the colonies were looser than the Ll colonies. They branched out more into the medium and continued to grow for a longer period. Like their parent bacterial strain the L type of culture obtained from *funduliformis* grew only under anaerobic conditions.

Another strain of *Bacteroides funduliformis* which was studied produced no L type colonies though the bacteria on certain media swelled up into large round bodies.

⁴ Dienes, L., *J. Inf. Dis.*, 1939, **65**, 24.

The isolation of the L type of growth was made possible both in the case of *flavobacterium* and of *funduliformis* by the elimination of viable bacteria by autolysis. The other cultures also in which the L type of colonies were observed, as in certain strains of *Bacillus coli*, *Bacillus influenzae* and of the gonococcus, possess a tendency to autolyze. In these cultures, however, the autolyzing was not complete and thus far all attempts to isolate the L type of growth have remained unsuccessful because the transplants were overgrown by bacteria.

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Relative Germicidal Action of Some Halogenated Phenols and Their Phenolates.

E. J. ORDAL. (Introduced by W. P. Larson.)

From the Department of Bacteriology and Pathology, University of Washington, Seattle.

Klarmann, Shternov and von Wowern¹ showed that the substitution of one, 2, and 3 atoms of chlorine into the phenol molecule led to a progressive increase in the germicidal action of the resulting compounds. Unfortunately, at the same time the solubility of the phenolic compounds decreased markedly with substitution. The sodium salts of these halogenated phenols possess a considerably greater solubility than the phenols themselves, but there is no explicit information available in the literature as to the actual germicidal power of the phenolates.

Tilley and Schaeffer² showed that the germicidal efficiency of phenol decreased with an increase in percentage of sodium hydroxide added until the percentage of sodium hydroxide equalled or slightly exceeded that required to neutralize the phenol. Phenol is a weak acid with the acid dissociation constant $K'_a = 1.06 \times 10^{-10}$ at 25°C.³ Consequently a completely neutralized solution of phenol will be strongly alkaline, and an attempt to evaluate the actual germicidal action of the phenolate is complicated by the alkalinity of the solution. The substitution of chlorine atoms into the phenol molecule increases

¹ Klarmann, E., Shternov, V. A., and von Wowern, J., *J. Bact.*, 1929, **17**, 423.

² Tilley, F. W., Schaeffer, J. M., *J. Agr. Res.*, 1931, **43**, 611.

³ *International Critical Tables*, prepared by National Research Council of the U.S.A., 1929.