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Further Observations on the L Organism of Klieneberger.

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The L organism which Klieneberger¹ isolated from the cultures of *Streptobacillus multiformis* and more recently directly from pathological lesions of rats presents many characteristics which are not observed in bacteria. It has a marked similarity to a group of microorganisms, the main representative of which is the causative agent of pleuropneumonia bovis. The relation of this group to the well characterized classes of microorganisms is obscure. The L organism starts to grow in very small units which pass readily through a coarse bacterial filter. Later the small forms develop into large yeast-like bodies. Probably the small bodies are reproduced by the disintegration of the large forms. In all stages of development the organism is very fragile and its form can be seen only by using special technic. Since pleuropneumonia-like organisms occur in different animals² and probably also free in nature,³ their occurrence in rats is not unexpected. However, it is very surprising to find such organisms regularly in the cultures of a bacillus.

Klieneberger regards the connection between the L organism and streptobacillus as a symbiosis. The two main arguments in favor of this supposition are: (1) the striking differences between the L organism and the bacillus; (2) the constancy of the properties of

¹ Klieneberger, E., *J. Path. Bact.*, 1935, **40**, 93; 1936, **42**, 587; *J. Hyg.*, 1938, **38**, 458; Klieneberger, E., and Steabben, D. B., *J. Hyg.*, 1937, **37**, 143.

² Shoentensack, H. M., *Kitasato Arch. Exp. Med.*, 1934, **11**, 277; 1936, **13**, 175 and 269) (Quoted after Klieneberger).

³ Seiffert, G., *Zbl. Bakt. I. O.*, 1937, **130**, 337; Oerskov, J., *Zbl. Bakt. I. O.*, 1938, **141**, 229.

THE L ORGANISM OF KLIENEBERGER

the L organism which in several years of cultivation never reverted to bacterial forms. The occurrence of L organisms without bacteria in animal lesions and the observation that the L organism forms mixed cultures with bacillus tetanus and certain other bacteria, give further support to the hypothesis that the L organism is a symbiont of the bacillus.

The observations which are here reported do not agree with this supposition. In the colonies of the streptobacilli the characteristic swollen forms do not develop as a separate growth admixed with the bacteria but are produced by the swelling up and transformation of the streptobacilli themselves. These large forms persist for a while or soon degenerate, but neither in the original cultures nor in transplants do they show any signs of multiplication. A transplant from a colony consisting mostly of the large forms gives a pure culture of the bacilli. The L organisms start to grow in the bacterial colonies after 48 hours or more of incubation in the form of a few secondary colonies attached to and partly embedded in the agar—and can be easily transplanted from these. The appearance of the L organism in transplantable form in the streptobacillus colonies is similar to the appearance of a colony variant.

Recent observations made with a freshly isolated strain of the streptobacillus strongly suggest that the L organism is actually a variant or growth form of the streptobacillus. The L organism was isolated from the secondary colonies of the streptobacillus. From the second transplant on, no streptobacilli grew in the cultures of the L organism on agar. This same strain of L organism was used for morphological studies and daily for 2 months numerous microscopical preparations were made from the cultures. No bacillary forms were visible in these preparations. As the bacillus grows faster and produces larger colonies than the L organism it is improbable that it would persist in latent form together with the L organism. However, on 3 different occasions small blocks of agar covered with colonies of L organism were put into nutrient broth containing boiled blood and ascitic fluid. For the first few days the L organism was present in the broth in pure culture, but after 3 days or more streptobacilli invariably appeared. Before the third experiment the L organism was consecutively transplanted 5 times from isolated colonies. Four of 6 broth tubes inoculated from this purified culture of L organism were found to contain streptobacilli, while 2 were contaminated during the successive transplants.

It should be mentioned that formerly strains of L organism directly isolated from the lungs of rats were maintained in the same

THE L ORGANISM OF KLIENEBERGER

kind of broth, but in these cultures the streptobacilli never appeared. Klieneberger recently pointed out that the strains directly isolated from the lesions are different both in their morphology and serological specificity from the strains isolated from the streptobacilli. The latter strains are serologically similar to the streptobacilli. Thus, the failure to cultivate the streptobacilli from the strains directly cultured from the lesions is not unexpected. Furthermore, the marked difference between the 2 groups of L strains in itself is rather against the symbiosis hypothesis. It is improbable that the symbiosis would so profoundly alter the properties of the strains.

The occurrence of such remarkable variation as the L organism in bacterial cultures is of considerable importance. For this reason caution is necessary in interpreting the observation. In a subsequent report observations will be presented and discussed showing that various groups of bacteria show more or less similar phenomena, thus supporting the conclusion that the L organism is a variant of the bacillus rather than a symbiont.