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Reproduction of Bacteria from the Large Bodies of *B. funduliformis*.^{*†}

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The significance of large round bodies which develop in various bacterial cultures was discussed in a preceding paper.¹ The germination of the large bodies usually produces a peculiar growth which in appearance and morphology is similar to the pleuropneumonia group of organisms. Observations of certain cultures suggest that this is not the only way in which the large bodies germinate; they may reproduce also bacteria of usual shape and size. The development of bacteria inside the large bodies was clearly visible in one instance in a colon bacillus culture.² The observation of a *B. funduliformis* strain in which this process occurred regularly and could be reproduced at will allowed a more thorough study of it.

Transformation of bacteria into large swollen forms is a characteristic property of *B. funduliformis*. In the first strain which was studied, the germination of the large bodies produced pleuropneumonia-like colonies which could be propagated in pure culture. In a second strain, under certain conditions, all bacteria swell up to large bodies, but these gave only bacterial growth in transplants. The method of derivation of the bacteria from the large bodies was not definitely established, but preparations made a few hours after transplantation suggested that the bacteria developed inside of the large bodies and were freed by their disintegrations. A third strain presented special advantages for the study of the development of large bodies. The cultures of this strain on

solid media consisted of small regular bacilli. In liquid media most of the bacilli swell up into large bodies. Bacillary forms were seen only rarely in broth cultures 36 hr old. After further incubation the large bodies disappeared and the culture consisted again of regular bacilli.

This strain grew very well on agar blocks under a coverslip surrounded by paraffin, anaërobiosis being obtained by inoculating a corner of the agar with staphylococci. On such agar blocks inoculated with 36-hr broth cultures only the large bodies were visible during the first few hours of incubation. After 4 to 6 hr, pleuropneumonia-like growth started from some of the large bodies. These were less refractile than the others and in stained preparations remained paler. Most of the large bodies developed into bacterial colonies. This occurred in various ways. A small sized large body may elongate itself to bacillary form or take up an irregular angular shape which by division falls into bacilli. Some of the large bodies fractioned into 2 or 4 parts and these developed into regular bacteria. The majority of the large bodies developed in a different way. They increased first in size and density and after 5 to 6 hours' incubation developed many prominences, taking up an irregular mulberry-like shape. The prominences developed to bizarre-shaped branching filaments which by fractionation produced regular bacilli. The filaments started in many directions from the large body sometimes producing a polyp-like structure. In stained preparations it may often be seen that the mulberry shape is produced by the fractionation of the contents of the large body into granules. These granules grew out into the branching filaments. Occasionally regular shaped bacilli developed inside of the large bodies and were freed by their disintegration.

The mulberry-shaped structures consisting

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¹ Dienes, L., *J. Bacteriology*, 1942, **44**, 37.

² Dienes, L., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 773.

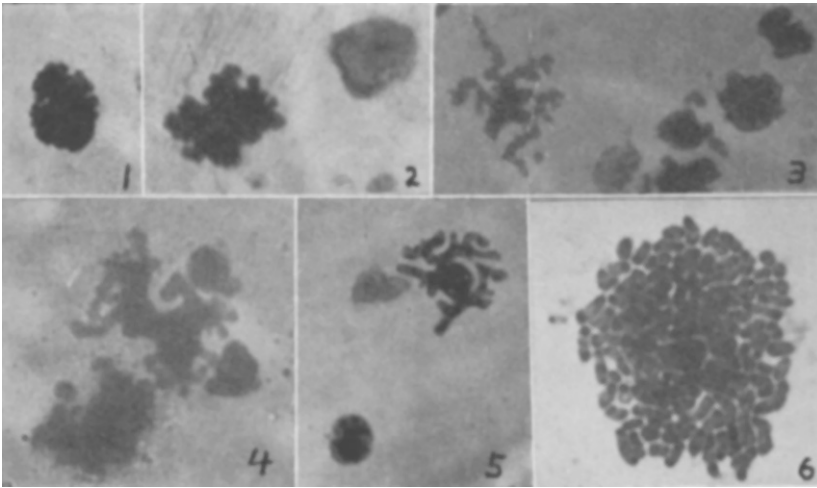


FIG. 1.

Photographs 1 to 6 show consecutive stages of the development from large bodies into bacterial colonies. Some of the large bodies in the photographs did not develop and show their original appearance. Photographs 1, 2, 3 and 5 were made from impression preparations stained with Giemsa following Klieneberger's³ agar fixation technic; 4 was made from a stained agar block and 6 from an impression preparation of an agar block stained with safranin.

of granules which develop to branching filaments are in some respects similar to the pleuropneumonia-like growth, but the filaments do not grow into the agar and within a few hours develop into regular bacilli. These structures probably represent unstable transitional forms between the pleuropneumonia-like and the regular bacterial growth. The strain has a tendency to grow in both forms on solid media.

The successive appearance of large bodies and regular bacterial forms in the cultures of our second and third strains indicates that

reproduction by means of the large bodies occurs regularly under certain conditions. The observation of a similar process in a colon bacillus culture already has been mentioned. It was claimed repeatedly in the literature that the large bodies were organs of reproduction but this conception did not gain recognition because the observations could not be reproduced at will and the pictures published were not regarded as decisive. The observations with our third strain seem to give definite evidence that the large bodies take part in the reproduction. The dual potentiality in the development of the large bodies remains an interesting problem.

³ Klieneberger, E., and Smiles, J., *J. Hygiene*, 1942, **42**, 110