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Chromatin Structures Suggesting a Nuclear Apparatus in the Large Bodies of *B. funduliformis.**†

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Ledingham[1] indicated the presence of "nucleus like" chromatin bodies in the large swollen forms of the organism of bovine pleuropneumonia. Klieneberger and Smiles[2] and Klieneberger[3] studying the mode of reproduction in the pleuropneumonia group observed that the chromatin divides and is distributed into the new cells. This chromatin gives the Fuelgen reaction indicating the presence of nucleic acid and Klieneberger attributes nuclear character to this material.

We have seen exactly similar chromatin material in the large bodies of various bacterial species.[4] We have been able to study it best in 3 strains of *Bacteroides funduliformis* because of the regularity with which these strains produce large bodies[5] and because of the excellent staining of their chromatin material. Pleuropneumonia-like (L) colonies frequently appeared among the ordinary bacterial colonies of 2 of these strains.

The present report deals with studies made on 1 of these strains which we have designated as Strain No. 132. This strain has been carried through more than 50 subcultures and continues to produce L variants in abundance. These L variants have been isolated in pure culture. They did not revert to their parent bacterial form throughout 30 subcultures on agar. They were strictly anaerobic, just as their parent bacterial forms.

The chromatin in the bacilli and in the L forms is best seen in preparations made by a modification of Klieneberger's agar fixation technic.[2] Agar blocks bearing the organisms are inverted onto cover slips and fixed for several days with Bouin's solution. The agar is then peeled away and the cover slip rinsed, stained with Giemsa, differentiated with ascitic fluid and mounted in balsam. The cover slip should never be allowed to dry until just before mounting, as shrinkage makes it impossible to see the precise distribution of chromatin within the organisms.

In broth, Strain 132 grows in the form of regular bacilli which swell up after 9 to 20 hours into large round bodies. The further development of these large bodies is best followed by transferring them to ascitic agar plates on which they produce either a bacterial or a pleuropneumonia-like colony. In a previous note[5] we described the fractionation of some of the large bodies into bacilli or the extrusion of multiple filaments which then segmented into bacilli. From other large bodies, small granules were observed to grow out and these developed into pleuropneumonia-like colonies.

With more experience in staining, it was seen that the large bodies contain deeply stained chromatin material embedded in a lightly stained material which may be most conveniently designated as cytoplasm. As illustrated in the photographs, the chromatin may be present in the form of discrete granules, in thread-like branching masses or in a single dense mass. When the large bodies reproduce ordinary bacilli, it was seen that they extrude tongues of "cytoplasm" and the chromatin grows out into these cytoplasmic filaments, which then segment to yield the

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1 Ledingham, J. C. G., *J. Path. Bact.*, 1933, **37**, 393.

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

3 Klieneberger, E., *J. Hygiene*, 1942, **42**, 485.

4 Dienes, L., *J. Bact.*, 1942, **44**, 37.

5 Dienes, L., and Smith, W. E., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 297.

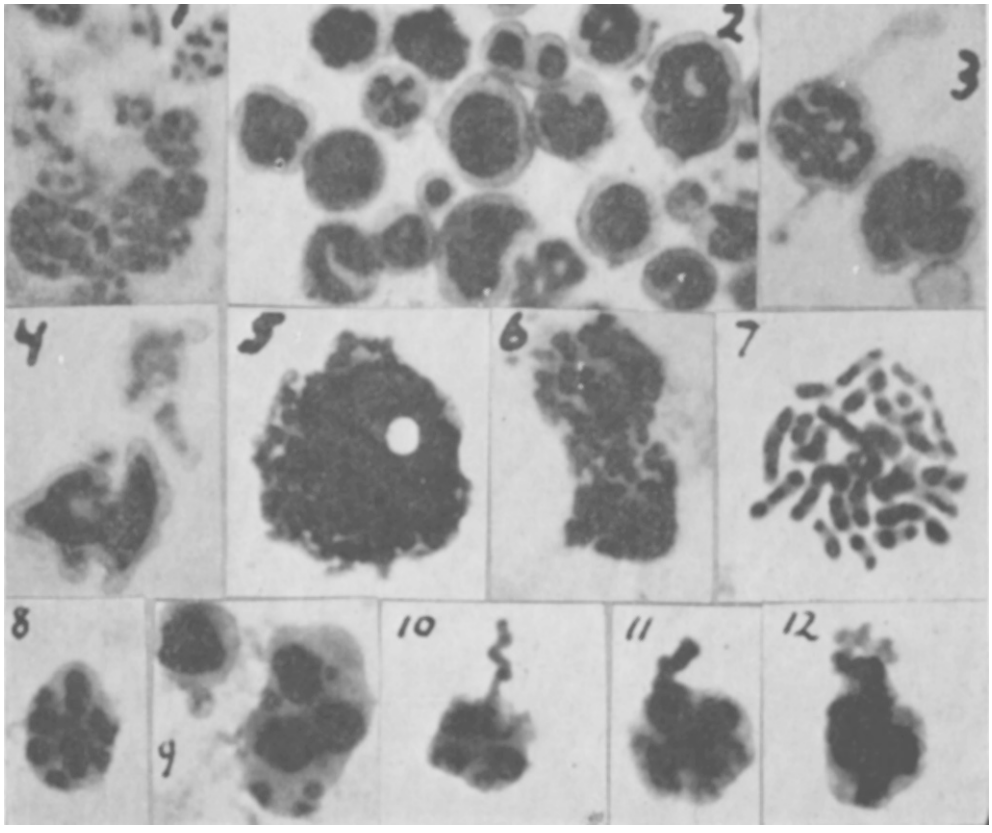

FIG. 1.

All photographs were made from Giemsa-stained preparations with high magnification (3000 ×). 1. Large bodies in a 24-hr broth culture. The chromatin is present in discrete granules. 2. Same culture as 1 after 6 hrs incubation on ascitic agar. The chromatin is in bands or condensed to compact masses. 3. Same preparation as 2. The chromatin is present in bands. The indentation in one of the large bodies indicates its development from two bacteria. See following note. 4. Same preparation as 2. The large body is deformed and begins to extrude bacterial filaments into which the chromatin is growing. 5 and 6. Fractionation of the chromatin before the large bodies disintegrate into bacteria. The large bodies are increased in size. 6½ hrs incubation on ascitic agar. 7. Same preparation as 5 and 6. Bacteria produced by disintegration of the large body. Every bacterium has one or more chromatin granules. 8 and 9. Large bodies with chromatin condensed into a few large masses. 3 hrs incubation on ascitic agar. 10, 11, 12. Growth of pleuropneumonia-like colony starting from the large chromatin granules. 3 hrs incubation on ascitic agar.

bacilli. The development of bacilli is often preceded by the breaking up of the chromatin into granules which are distributed into the bacteria. Frequently, the "cytoplasm" fractions into masses around the chromatin granules and bacilli are thus formed within the large body. The chromatin is present in the bacilli as small discrete granules or elongated masses. When the large bodies reproduce pleuropneumonia-like colonies, there is no extrusion of cytoplasmic filaments and the chromatin is seen in 1 or several large masses. These masses extrude fine chromatin filaments which become nodular and give rise to the pleuropneumonia-like growth.

These observations support the view that the chromatin has a nuclear character and that it is connected both with the reproduction of bacteria and of the L type of growth.