
The Formation and Development of L-Forms in Certain Bacterial Species

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The purpose of this work was to demonstrate the nuclear development in large bodies of *Bacillus proteus*. The original culture was obtained from Dr. Lewis Dienes in March of 1952. It had been carried successively through many sub-cultures without a loss in ability to produce large bodies. It was typed as a *Bacillus proteus* by Dr. Dienes, but in the 6th edition of Bergey's Manual it is classified as *Proteus vulgaris*. Under proper environmental conditions it has never failed to produce large forms (4).

The organisms were grown upon stock culture agar to which was added 10 units of penicillin G per ml. Various concentrations of penicillin ranging from 1 to 100 units per ml. of media were tried and the optimum for production of the large bodies appeared to be around 10 units per ml. (5, 6). Beef and horse serum agars incorporating similar amounts of penicillin did not appreciably increase or decrease the production of the large bodies and the only variation to be found in the use of the serum agars was represented in the colony morphology (7).

Three stains were used to demonstrate nuclear bodies. These were a modified Robinow's stain (3), Crystal Violet (2), and a Reduction-Oxidation stain (1). All the stains gave identical results but the demonstration of the nuclear bodies was most satisfactorily observed in the Reduction-Oxidation stain. A series of three slides was prepared at intervals throughout a 144 hour period. Large body production was noted to occur up to 120 hours, but appeared to have terminated by 144 hours.

The cycle of production of large bodies does not appear to be uniform. They may be produced from cells having undergone normal division for several hours or the large bodies may occur upon the first division and lastly, the large bodies may be formed from a cell not having undergone any division at all after transfer. During the first six hours of growth, it is possible to observe all phases of nuclear development. The optimum time for the production of the large bodies varies from 4 to 16 hours. *In vivo* studies indicate the total time for the formation of a mature large body to be approximately two and one-half hours. The control, grown

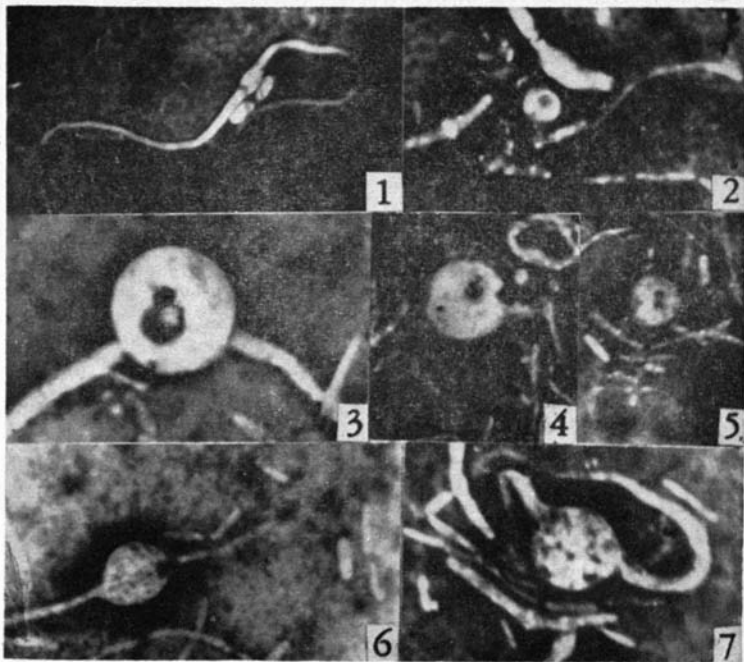


FIGURE 1. Concentration of Nuclear Bodies in Young Large Forms. Approx. 2500 X.

FIGURE 2. Massing of Nuclear Material in Young Mature Large Bodies. Approx. 2500 X.

FIGURE 3. Complete Development of Large Body with Concentrated Mass of Nuclear Material Intact. Approx. 3500 X.

FIGURE 4. Formation of Individual Nuclear Bodies from the Large Nuclear Mass. Approx. 2500 X.

FIGURE 5. Apparent Mitosis of Nuclear Material in Large Body. Approx. 2500 X.

FIGURE 6. Division of Individual Bodies from Large Nuclear Mass. Approx. 2500 X.

FIGURE 7. Complete Dispersal of Nuclear Material from Large Nuclear Body. Approx. 2500 X.

upon stock culture agar without any penicillin, did not produce any large bodies during the entire period of observation and appeared to follow the normal nuclear developmental patterns.

The photographs were made from a 12 hour old culture using the Reduction-Oxidation method of staining. Oil immersion was used for all observations and the photographs were made with Kodak Panatomic X. An attempt was made to use phase microscopy for the identification of the nuclear components but the results were highly inconclusive.

The Figures are arranged in an order which seemed to correspond to the order of nuclear development. This arrangement was determined by two methods:

1. The relative increase and subsequent development of the large bodies.
2. Accepted patterns for known nuclear development.

As stated before, since there is no known uniform development of large bodies, it has been impossible to follow them on a time interval basis. Size to a great extent is also highly misleading; therefore, it must be said that the arrangement is highly arbitrary.

Development of the nuclear bodies following the complete nuclear material dispersion (Figure 7) is found to proceed the same as does the normal type of development. The extruded filaments, consisting of apparently normal bacillary forms, may or may not produce other large bodies during subsequent growth. Autolysis, accompanied by the liberation of the inclusion material, occurs approximately 3 to 5 hours after formation.

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