THE INFLUENCE OF PENICILLIN ON LARGE BODY PRODUCTION BY LUMINOUS BACTERIA[1]

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The normal occurrence of large bodies in young cultures of the marine luminous bacterium *Achromobacter fischeri* has been recently observed (Johnson and Gray, 1949). This report deals with the influence of penicillin, which in appropriate concentrations has been shown to favor the production of large bodies, as well as L_1 types of cells and colonies, both in common bacteria such as *Escherichia coli* or *Proteus* and in pleuropneumonialike organisms (Dienes, 1947, 1948, 1949; Tulasne, 1949*a*,*b*,*c*).

METHODS AND RESULTS

The strain of *A. fischeri* was the same as that used in the previous study (Johnson and Gray, 1949), likewise the methods of maintenance and cultivation for nuclear staining by the HCl-Giemsa technique. The strain has been kept here in active or in stored cultures for about 17 years. Four-hour plate cultures at 28 C on brain heart agar plus 3 per cent NaCl were used as sources of inocula for a second set of plates, of the same medium to serve as controls or with the addition of sterile penicillin solution just before pouring. Inoculations were also made from the 4-hour cultures to 3 per cent NaCl nutrient agar containing 10 per cent horse serum, with and without penicillin.

Concentrations of penicillin of less than 50 units per ml had little effect beyond a slight retardation in growth and luminescence and a slight increase in numbers of large bodies. The stained specimens corresponded in appearance to those described earlier (Johnson and Gray, 1949). With 200 to 500 units of penicillin per ml, however, large bodies were produced in great abundance within 2 hours of incubation, and for the next several hours the cultures consisted mostly of long, filamentous cells and numerous large bodies (figure 1). No luminescence was visible at this stage. On further incubation for half a day or longer, varying somewhat in repeated experiments, the filaments and large bodies were gradually replaced by the characteristic small rods, and the cultures always gradually became luminous. Whether the luminescent cultures arose from a few resistant rods or normal long cells, or whether they arose from a transformation of large bodies, is not certain, but it is clear that nonluminous cultures of mostly large bodies and long filaments may later show luminescence associated with the familiar morphological units. By analogy with other species, it would seem probable that some of the large bodies do, indeed, give rise to normal luminous cells.

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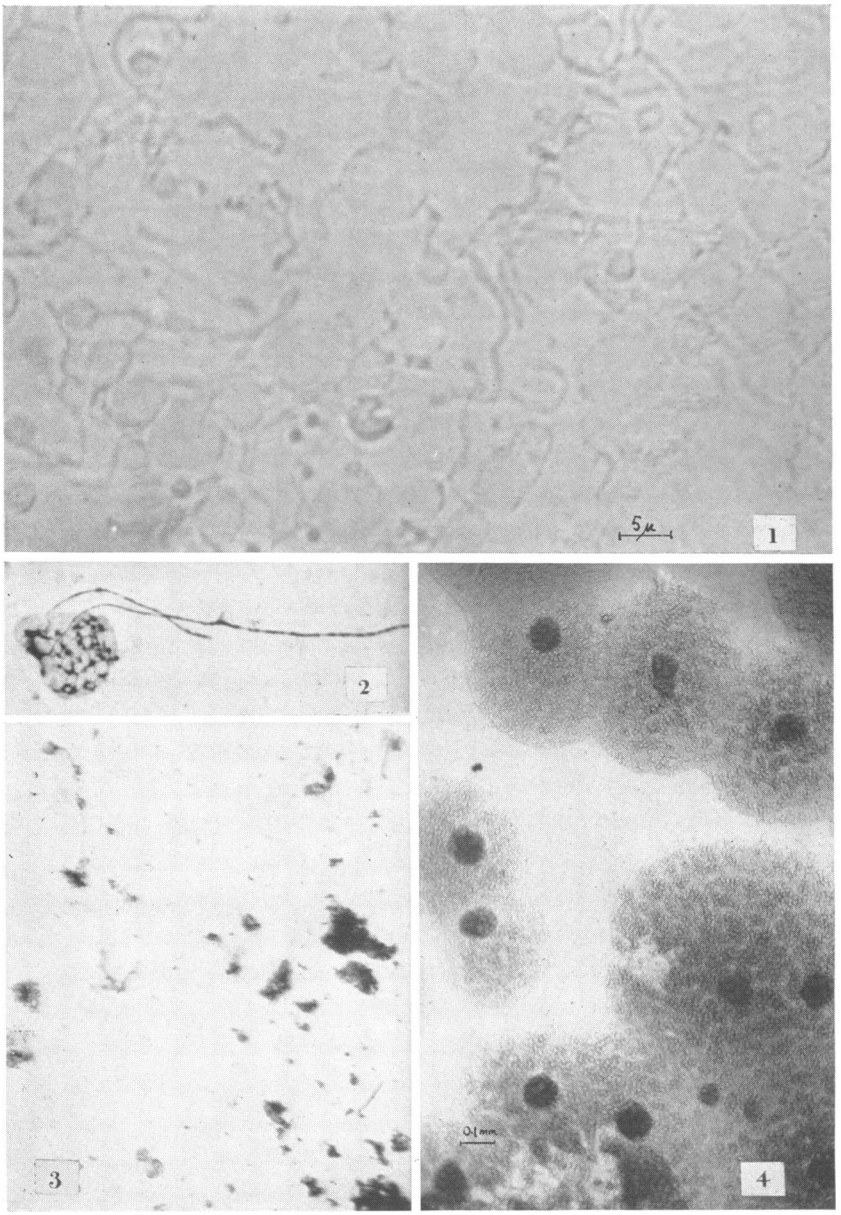

Figure 1. A. fischeri, unstained, living cells and large bodies on the surface of brain heart agar containing 300 units of penicillin per ml, incubated 5 hours at 28 C, followed by 4 hours at room temperature. Magnification 1,450×.

Figure 2. Single large body of *A. fischeri* stained for chromatin by the osmium-HCl-Giemsa method from a brain heart agar plate with 300 units of penicillin per ml, incubated 7 hours at 28 C. Magnification 1,450×.

Figure 3. L_1-like microcolonies of *A. fischeri* on a plate similar to that of figure 2 but incubated for 9 hours at 28 C. Stained by the osmium-HCl-Giemsa method. Magnification 1,450×.

Figure 4. Dark-centered colonies of *A. fischeri* on nutrient agar plate with 10 per cent horse serum and 300 units of penicillin per ml, incubated initially for a few hours at 28 C and subsequently for 3 days at room temperature. Magnification 45×.

Further suggestive evidence that this occurs was found in stained preparations. In specimens from cultures containing 300 units of penicillin per ml, incubated until the large bodies appeared to have reached maximum size, usually within 9 hours at 28 C, there seemed to be a fairly continuous gradation of stages between complete large bodies with apparently amorphous or scattered chromatin, to examples of microscopic L_1-like colonies to which they might have given rise. Figure 2 shows an unusually clear example of a large body containing numerous small units of chromatin, which were possibly destined for release through autolysis of the body itself and later development into normal rods. Figure 3 illustrates what appears to be the remnants of a large body at one end of a shriveled filament, with a group of chromatin units at the other. Additional groups of such units are apparent in the same field. The supposition that they represent a stage toward the formation of normal cells from large bodies is strengthened by the fact that this stage precedes by a relatively short time the development of luminescence with the presence of normal rods.

With 1,000 units of penicillin per ml, large bodies formed both rapidly and abundantly, but no luminescence appeared. A few nonluminous colonies usually developed and, when transferred to ordinary media, growth and luminescence occurred normally.

In concentrations of 5,000 to 10,000 units of penicillin per ml, apparently all the cells in the inoculum underwent transformation into large bodies, which, however, remained much smaller than those on media containing 300 to 500 units per ml. They were difficult to stain successfully and disappeared in from 7 to 12 hours. Microscopic study of specimens *in situ* indicated that, in the cases observed, they underwent a sudden, spontaneous autolysis.

In some cultures, well-defined small colonies with dark centers, resembling those of *Proteus* described by Dienes (1949), developed (figure 4) and luminesced. Light emission was again associated with the presence of normal rods, although the appearance of the colony was in marked contrast to the normally homogeneous colony of this species on ordinary media. Luminescent colonies resembling those pictured in figure 4, but less clearly defined, were also observed on soft agar with 10 per cent horse serum, with or without penicillin of 300 units per ml.

SUMMARY

In concentrations of 300 to 500 units per ml, penicillin greatly increases the number of large bodies produced by *Achromobacter fischeri* on 3 per cent NaCl brain heart agar. Observation of living and stained specimens gave suggestive evidence that the large bodies gave rise to L_1-like microcolonies and to normal, luminescent rods. High concentrations of penicillin prevented luminescent cultures and led to abundant production of large bodies, of smaller size, which later autolyzed.

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