THE REVERSION OF THE L FORM OF PROTEUS MIRABILIS INTO THE ROD FORM

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Several Proteus strains have been reported to form L type colonies when inoculated onto penicillin agar (Dienes, 1949; Tulasne, 1949; Medill and Hutchinson, 1953). These colonies are characterized by a dense center imbedded in the agar and a periphery on the surface of the medium. They contain elements which are spherical in shape and vary in diameter from 0.2 to 7 µ or more. Tulasne (1953) designates the smaller forms as elementary bodies and the larger ones as large bodies. In the present paper the term “L form” will be used to refer to any element found in an L type colony in order to distinguish these forms from the large bodies which are formed in many bacterial species under a variety of conditions. The identity of these latter spherical forms with L forms is not established. The present paper presents a description of the reversion of the L forms into bacilli, illustrated by consecutive photographs of the same living cells undergoing the entire process.

METHODS

The organism used in this investigation was Proteus mirabilis, strain K, which was kindly supplied by Dr. James C. Kakavas of the University of Delaware. This strain is an active swarmer. The medium used to support the growth of L type colonies was prepared by solidifying tryptose blood base broth (Difco) with 1.1 per cent agar. After autoclaving, 200 units of penicillin per ml and one per cent “PPLO” serum (Difco) were added.

Slide cultures were prepared by removing a block of agar containing L type colonies and inverting and smearing this onto tryptose agar without penicillin. A block of this latter agar containing L forms was removed then to a sterile slide, covered with a sterile coverslip, and sealed with vaspar leaving air space between the vaspar and agar block. It was found that the addition of a drop of broth between the agar and coverslip greatly improved the viability of the L forms. When penicillinase was used, a drop of broth containing one per cent “penase” (Difco) was placed between the agar and coverslip. These cultures were incubated at room temperature on the stage of the microscope, and the cells were observed continuously as they developed into bacilli. Time-lapse motion picture photographs were recorded on Cine-Kodak super XX film with a time interval of 20 seconds between exposures. A Spencer 95x oil immersion dark M phase contrast objective was used with a 25x eye piece.

RESULTS

Since small amounts of penicillin carried over from the inoculum conceivably might affect the development of the L forms into bacilli, penicillinase was included in some of the preparations. Under any conditions, the larger L forms were the only ones observed to return to bacilli. The transitional L forms tend to branch and become actively motile in the presence of abundant moisture. With the restricted moisture necessary for successful time-lapse photography, branching is inhibited but still observable. A typical reversion is illustrated in figures 1 to 13. As seen in figures 2 to 4 the L forms appear to initiate division. However, by the end of about two hours it is evident that division is not to be completed, but rather an elongation and branching of the cells occur (figures 5 to 13). Several divisions of the elongated structure ultimately break it up into rod forms. Subsequent development of the bacilli so formed differs, depending on whether or not penicillinase is present. With penicillinase the bacteria continue to multiply by binary fission in the small rod form. Swarm cells are seldom observed. Without penicillinase the small rods go through a period of active division and motility, which is followed by a period during which the cells appear to become attracted to each other.

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Figures 1 to 13. Development of L forms into bacilli. The inoculation was made from four day old L type colonies. A dark M contrast objective was used, and the prints are negatives of the original image.

Figure 1—30 minutes after inoculation
Figure 2—70 minutes after inoculation
Figure 3—83 minutes after inoculation
Figure 4—109 minutes after inoculation
Figure 5—122 minutes after inoculation
Figure 6—135 minutes after inoculation
Figure 7—148 minutes after inoculation
Figure 8—160 minutes after inoculation
Figure 9—173 minutes after inoculation
Figure 10—186 minutes after inoculation
Figure 11—199 minutes after inoculation
Figure 12—212 minutes after inoculation
Figure 13—225 minutes after inoculation
Small and large clumps of bacteria are observed for about an hour. Swarm cells then appear swimming about freely. Within the next several hours large globular swellings are developed in the center of these long rods. Motility continues and the culture consists almost entirely of such swarm cells with central swellings 16 hours after inoculation. If the slide culture is overcrowded, development stops; but if the growth is transferred to new media, the ends of the swarm cells can be observed to fragment into small rods while the central portion becomes vacuolated and disintegrates. This sequence of events has been observed many times. If the bacilli resulting from the reversion in the presence of penicillinase are inoculated onto penicillin agar, L type colonies are formed readily. Many efforts have been made to induce the bacilli resulting from reversion in the absence of penicillinase to form L type colonies. These efforts have uniformly failed unless the culture is allowed to grow in the rod form through an additional transfer before reincoculation onto penicillin agar.

DISCUSSION

Freundt (1950) and Pulvertaft (1953) both describe reversion of other strains of Proteus L forms by a process similar to that described above. Tulasne (1953) describes four methods of the reversion of L forms of Proteus to bacilli. The L form ruptures liberating rods, the L form divides longitudinally into five or six filaments, the L form grows into a fusiform cell from which rods eventually break off, or it develops by a method which appears to be similar to that illustrated in the present paper. None of these workers has published consecutive photographs of the same living cells to illustrate these processes, and therefore it is difficult to compare in detail their observations with the present ones.

The development of Proteus large bodies (as distinguished from L forms) into bacilli has been illustrated by time-lapse photography of living material by Stempen and Hutchinson (1951). The large body becomes irregular and divides into several bacteria, a process which is very different from the elongation and branching of the L forms. Dienes and Weinberger (1951) state that the L forms of Proteus revert to bacilli in the same manner as do the L forms of Bacteroides. However, the description (Dienes and Smith, 1944) deals with the development of large bodies which have formed from the bacilli not with the L forms found in the L type colonies. This process in Bacteroides is illustrated by photographs of living cells and is similar to that described by Stempen and Hutchinson (1951) for Proteus large bodies, but differs from the method described here for Proteus L forms.

Although penicillin initiates both large body and L type colony production, almost all the bacilli will form large bodies, but less than one per cent forms L type colonies (Medill and Hutchinson, 1953). Tulasne (1951) and Dienes (1949) state that L type colonies are formed from large bodies. Although their observations are very convincing, they are not illustrated by consecutive photographs of the same large body undergoing this entire transformation. Klieberger-Nobel (1951) describes the initiation of L forms by the release of small granules from the rods, but she does not state whether these granules initiate L type colonies.

Dondero and Zelle (1953) have demonstrated large body formation to be clonal in Azotobacter agit. On the other hand, L type colony formation has been shown to be nonclonal (Medill and Hutchinson, 1953).

Since the reversion of large bodies and L forms into bacilli differs, and since a connection between these two forms has not been definitely established, it would appear to be desirable to distinguish between them until further information is obtained as to their respective natures.

SUMMARY

The reversion of the L forms of Proteus mirabilis into bacilli was recorded by time-lapse motion picture photography using the dark phase contrast microscope. The spherical L forms elongate, branch, and break up into small rods. Branching is stimulated by moisture between the agar and cover slip. If penicillinase is present, the rods resulting from the reversion form only an occasional swarm cell, while in the absence of penicillinase abundant swarm cells are formed which develop central globular swellings.

ADDENDUM

After this paper was submitted for publication, a paper by von Prittwitz und Gaffron (Naturwissenschaften, 40, 590, 1953) appeared. The photographs in this paper show reversion of spherical
forms of *Proteus vulgaris* to rods in a manner entirely similar to that shown in the present paper. The strain used by von Prittwitz und Gaffron, however, does not produce L type colonies, and their photographs are of spherical forms derived directly from rods on penicillin agar rather than from L type colonies. The photographs accompanying the present paper are of elements removed from typical macroscopic L type colonies which have been growing on penicillin agar for four days. The difference between those penicillin induced spherical forms which will reproduce in the L form and those which will not remains to be clarified.

REFERENCES


