DEMONSTRATION OF A CELL WALL IN THE LARGE BODIES OF PROTEUS VULGARIS

HENRY STEMPEN

Department of Bacteriology and Immunology, Jefferson Medical College of Philadelphia, Philadelphia, Pennsylvania

Received for publication January 17, 1955

The ability of penicillin to induce morphological changes in various strains of Proteus is already well known from the investigations of Örskov (1947), Lahelle (1948), Levaditi and Henry (1948), Dienes (1949), Tulasne (1949a,b, 1950), Fleming et al. (1950), Freundt (1950), Pulvertaft (1953), and von Prittwitz and Gaffron (1953). The ease with which Proteus produces spherical forms, so-called "large bodies", leads one to inquire about the fate of the cell wall during this transformation. Such a change in shape may result from either the rupture of the cell wall followed by the extrusion of the protoplasm or a change in the rigidity of the cell wall. The present investigation deals with a description of the morphological changes which occur in Proteus vulgaris strain OX19 when grown in the presence of penicillin and with attempts to demonstrate a cell wall in the different morphological forms.

MATERIALS AND METHODS

Large body formation was induced by growing Proteus vulgaris strain OX19 on nutrient agar and blood agar base containing 50 units of penicillin per ml of medium. The mode of formation and development of the large bodies was observed microscopically in slide cultures. A square on the surface of the medium was inoculated with a loopful of brain heart infusion broth culture. The broth culture was incubated at 30 C for 18 to 24 hours. The square was cut out and mounted between two sterile cover glasses. The preparation was sealed with sterile paraffin and incubated at room temperature on the stage of the microscope. Observations were made with the aid of a Spencer bright M phase contrast oil immersion objective.

Of the different cell wall strains found in the literature, those used by Knaysi (1941) and Robinow (1945) were employed in this investigation. Plate cultures were prepared by spreading 0.1 ml of the infusion broth culture over the surface of the agar. They were then incubated at 30 C. Squares of agar were cut and removed at intervals, and the organisms were stained by each technique.

In an attempt to demonstrate the cell wall by plasmolysis, the cells were first suspended in M sodium chloride solution in a small test tube for 5 minutes. Several loopfuls of the suspension were spread on a square of agar. After the moisture had been absorbed by the agar block, the latter was placed between two cover glasses and sealed with paraffin. Observations were made with the aid of a Spencer dark M phase contrast oil immersion objective.

RESULTS

Morphological changes. Large body formation occurs either by the appearance of a lateral bud-like structure (figures 1 to 4 and 11 to 14) or less often by the production of a terminal swelling. All but a few rods are transformed into large bodies, whereas in the absence of penicillin, only a relatively few large bodies are formed. If the large bodies which had formed on nutrient agar containing penicillin were allowed to remain on the original medium, they usually did not develop further, although an occasional one lysed. Those cells grown on blood agar base containing penicillin continued to increase in size and formed small protuberances on the surface (figures 5 to 7). These protuberances increased in length and appeared to subdivide into normal appearing rods (figures 8 to 10).

Cell wall stains. When this strain of Proteus was grown on blood agar base containing penicillin and stained by Knaysi's method, a dark blue wall and red cytoplasm could be observed (figures 15 to 19). After 24 hours' incubation the rods were very small, and a wall could not always be observed with certainty. In a number of cases (figures 32a and 32b), the cytoplasm was
Figures 1 to 19, 26, 28 to 30, 32a and 32b represent cells grown on blood agar base containing 50 units of penicillin per ml. Figures 20, 22 to 25, 27, and 31 show cells grown on nutrient agar containing the same amount of penicillin.

Figures 1 to 10. Formation and development of large bodies. The upper cell illustrates large body formation by a bud-like structure. The culture is $\frac{1}{4}$, $\frac{1}{6}$, $\frac{1}{12}$, $\frac{4}{12}$, $\frac{5}{12}$, 6, $6\frac{1}{2}$, $7\frac{1}{2}$, and 24 hours old; $\times$ 1,900.

Figures 11 to 14. Large body formation by the production of a lateral bulge (lower cell). The culture is $\frac{1}{6}$, $\frac{1}{12}$, 2, and 2$\frac{1}{2}$ hours old; $\times$ 1,750.

Figures 15 to 19. Stages in the formation and development of large bodies as demonstrated by Knaysi's cell wall stain. The culture is $\frac{1}{4}$, 1, 3, 4, and 5 hours old; $\times$ 1,750.

Figure 20. Cells from a 24 hour old culture stained by Knaysi's method. One of the rods has a typical dark outline, whereas the other rod and the large bodies show a diminished affinity for the dye; $\times$ 1,750.

found to be drawn from a fainter, outer structure. The blue outline followed the margin of the cytoplasm in this region.

When grown on nutrient agar containing penicillin and stained by Knaysi's method, the stages in the formation of the large bodies stained as those above. The large bodies at first also stained with a distinct blue outline. After 4 to 5 hours' incubation, however, samples taken from the same culture showed that the majority of the large bodies as well as many of the rods were surrounded by only a very faint blue outline (figure 20).

When the cells were stained by the Robinow technique, a dark purple outline surrounding a pale purple cytoplasm could be demonstrated in all of the morphologic forms (including the large bodies on nutrient agar containing penicillin) indicated in the previous sections except with the rods after 24 hours' incubation on blood agar base containing penicillin. In this case, the rods stained light purple, and a darker purple
outline could be distinctly seen to surround only a few.

The difference in the results obtained with the two staining methods on large bodies formed on nutrient agar containing penicillin is partially clarified by two simple experiments. Large bodies from a 24 hour old culture on this medium when fixed and mordanted according to Robinow's method but mounted with carbol fuchsin showed a typical cell wall stain. A dark blue outline surrounded the red cytoplasm. An identical picture resulted when fixation by Bouin's solution was followed by mordanting and staining by Knaysi's method.

Plasmolysis. The cytoplasm in the forming large bodies was most often shrunken inward from the outer edge of the bulged portion. In this way, the cytoplasm could be differentiated from the wall which presumably retained the original contour of the organism (figures 22 and 23).

Two types of large bodies could be distinguished when the cells were grown on nutrient agar and exposed to m sodium chloride. Type a (figure 24) showed the cytoplasm eccentrically placed and drawn back from a part or most of the wall. In type b (figure 25), a wall could not be differentiated from the cytoplasm which contained one or more dark, ring-shaped structures. Type a forms were more numerous than those of type b in young cultures (5 to 6½ hours); whereas the reverse was true in older cultures (23 hours). When the cells were grown on blood agar base containing penicillin, large bodies corresponding to those of type b were found only on rare occasions. The others were similar to type a. The cytoplasm in a number of cases was divided into two distinct portions (figure 26) or less frequently into three unequal portions.

Plasmolyzed large bodies when stained with Robinow's technique often presented an appearance similar to the type a forms (figures
27 to 30). The portion corresponding to the shrunken cytoplasm was surrounded by a dark purple outline which often appeared more dense at some points than at others. That portion corresponding to the wall was less intensely stained. Occasionally, the area between the cytoplasm and the outer margin of the wall also appeared lightly stained as though the wall had collapsed during fixation. Very often, however, evidence of an outer wall could not be found (figure 31).

Forms representative of stages in the development of large bodies into rods were more resistant to plasmolysis by m sodium chloride solution. The cytoplasm was, however, often observed to be shrunken from the end of a protrusion or filament which exposed a thin wall. Rods produced from the large bodies as well as those from the original inoculum were similarly difficult to plasmolysize. When plasmolysed, however, they appeared as the rod shown in figure 21.

**DISCUSSION**

Large body formation in *P. vulgaris* OX19 occurs in the same manner under the influence of penicillin as previously described under normal cultural conditions by Stempen and Hutchinson (1951). The lack of further development of the large bodies on nutrient agar containing penicillin is in agreement with reports on other strains of Proteus (Ørskov, 1947; Dienes, 1949; Tulasne, 1949a; Fleming et al., 1950; Pulvertaft, 1953; von Prittwitz and Gaffron, 1953) in which development into rods was obtained only when the spherical forms were transferred to media not containing penicillin. On blood agar base containing penicillin, however, the large bodies of *P. vulgaris* OX19 are capable of reverting to the rod form.

The results obtained with Knaysi’s method suggest that the large bodies formed on nutrient agar containing penicillin lose their cell wall substance. This apparent loss may be due to a change in the material at the outer region of the cytoplasm, for some of the evidence presented in this investigation indicates that this region is largely responsible for the “cell wall” staining by Knaysi’s technique. Although the properties of the cell surface may have altered slightly, a typical cell wall staining reaction may be obtained if the method of fixation is changed.

Phase contrast microscopic observations of *P. vulgaris* OX19 exposed to m sodium chloride solution show that the cytoplasm can be made to shrink away from a thin, outer structure. This latter structure behaves in the same way as the cell wall of other bacteria which have undergone plasmolysis (Robinow and Murray, 1953). Its identity with the cell wall may also be inferred from the results obtained with Robinow’s technique. By this method, both cytoplasmic membrane and cell wall stain, the former appearing more intensely colored than the latter (Welshimer and Robinow, 1949). The large bodies in the present investigation show a mass which is bounded by a dark purple outline. This mass appears to correspond to the shrunken cytoplasm as seen by phase contrast microscopy. That structure corresponding to the wall is lightly stained. It may be concluded that the structure which retains the original shape of the cell after plasmolysis is the cell wall.

**SUMMARY**

The presence of penicillin in solid culture media induces the formation of “large bodies” in cultures of *Proteus vulgaris* strain OX19. With nutrient agar no further development occurs. On blood agar base the large bodies develop protrusions which elongate and divide into regular rod forms.

Large bodies formed on each of these media were shown to have a cell wall both by staining methods and by phase contrast microscopy after plasmolysis. A similar wall-like structure was demonstrated in developing large bodies as well as in normal bacillary forms.

**REFERENCES**


