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Toxin Production and Morphological Changes of *Vibrio cholerae* in the Medium for Inducing Pleomorphism

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Abstract: Pleomorphism in *Vibrio cholerae* was induced by glycine under hypertonic conditions. Under these conditions, almost all of the organisms converted from a rod shape to a round shape with pleomorphism. However, RPHA (reversed passive hemagglutination assay) and rabbit skin reaction tests failed to detect toxin in the medium. These morphological changes were similar to those seen in cultures with shaking syncase medium at 30°C, in which the organisms released a large amount of toxin. It is suggested that this kind of morphology does not always accompany toxin excretion.

INTRODUCTION

The morphological changes of *Vibrio cholerae* during toxin production have been investigated by several workers (Chatterjee and Das, 1967; Kennedy and Richardson, 1969; Fernandes *et al.*, 1977; Fernandes and Smith, 1977; Iwanaga and Naito, 1979). However, their observations are not in agreement. In our previous report (1979), we suggested that the appearance of large spherocytes and membrane enveloped particles, possibly originating from the cell wall, might be related to the release of toxin. In the present study, we tried to induce pleomorphism of *V. cholerae* with a method other than the one used in the previous study, and assayed the amount of the toxin in cultures exhibiting pleomorphism.

MATERIALS AND METHODS

Bacterial strains: *Vibrio cholerae* 569B, a highly toxigenic, and *Vibrio cholerae* El Tor K-25, a non (or weakly) toxigenic strain, were used in this study.

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Culture to induce pleomorphism: A modified version of Kawata's method (Kawata and Inoue, 1963) was used. For each of the strains, a colony grown on meat extract agar was inoculated into nutrient broth (Difco), and incubated for 12 hours at 37°C. 1.0 ml of the cultured media was reinoculated into 9.0 ml of fresh nutrient broth and incubated for 4 hours at 37°C. The total amount (10 ml) of this 4 hour culture was then added to 90 ml of fresh nutrient broth containing 10% sucrose, 0.2% magnesium sulfate and 1% glycine, and cultured for an additional 4 to 5 hours. Samples for toxin titration and morphological study were obtained by centrifuging the cultured media at 8,000 g for 30 minutes. The supernatant was used for toxin titration studies, and the cell pellet was examined by electron microscopy.

Toxin assay: Supernatants were obtained by centrifugation of the culture, followed by filtration with a 0.45 micron millipore membrane. Dialysed culture filtrates were prepared by dialysis in cold phosphate buffer (0.1M, pH7.3) for 5 hours to remove sucrose. The buffer solution was changed every hour. The toxin in dialysed and undialysed culture filtrates were assayed by the reversed passive hemagglutination method (RPHA) (Holmes *et al.*, 1978) and the rabbit skin reaction test (Craig, 1965). The preparation of sensitized erythrocytes for RPHA was kindly supplied by Dr. Ohtomo and Dr. Muraoka, Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan.

Electron microscopic technique: The cell pellet obtained after culture centrifugation was treated by the same method in the previous paper (Iwanaga and Naito, 1979).

RESULTS

Morphological observations: By using the modified Kawata's method, almost all of the organisms converted from a rod shape, to a large, spherical shape with pleomorphism. This pleomorphism was observed in both strains 569B and K-25 (Photos. 1 and 2). A large number of membrane enveloped particles and some bulgings of the cell wall were also seen (Photos. 3 and 4). A moderately dense, crumb-like material was often seen in the cytoplasm of strain 569B (Photo. 5), but only rarely seen in strain K-25. This material was generally seen in the electron lucent space, and it consisted of a homogeneously dense substance which was enveloped by many ribosome-like particles (Photo. 6).

Toxin assay: The dialysed and undialysed supernatants of strain 569B, and the undialysed supernatant of strain K-25 were titrated for cholera toxin. The levels of toxin in all of the samples were less than 1.0 ng/ml. No reaction was seen in the rabbit skin test.

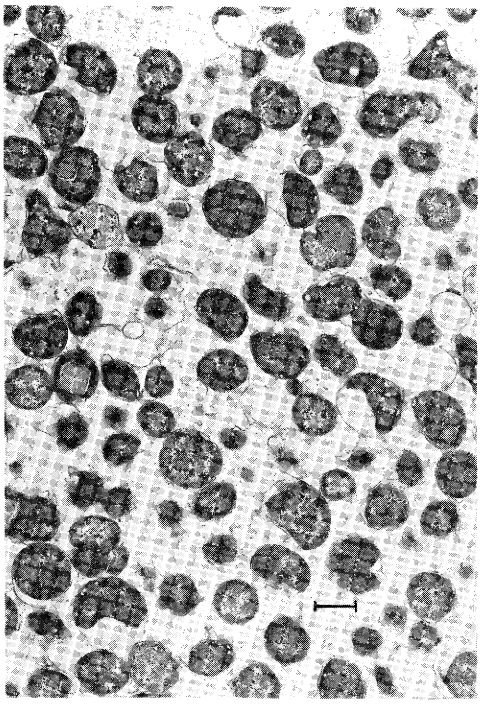


Photo. 1. (Strain 569B)

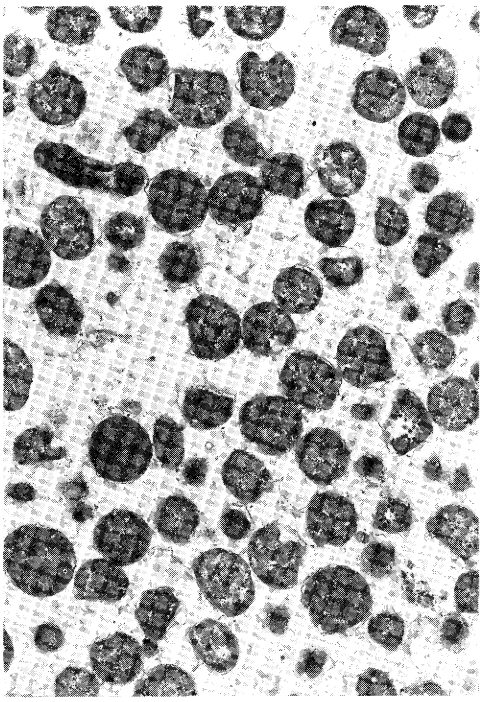
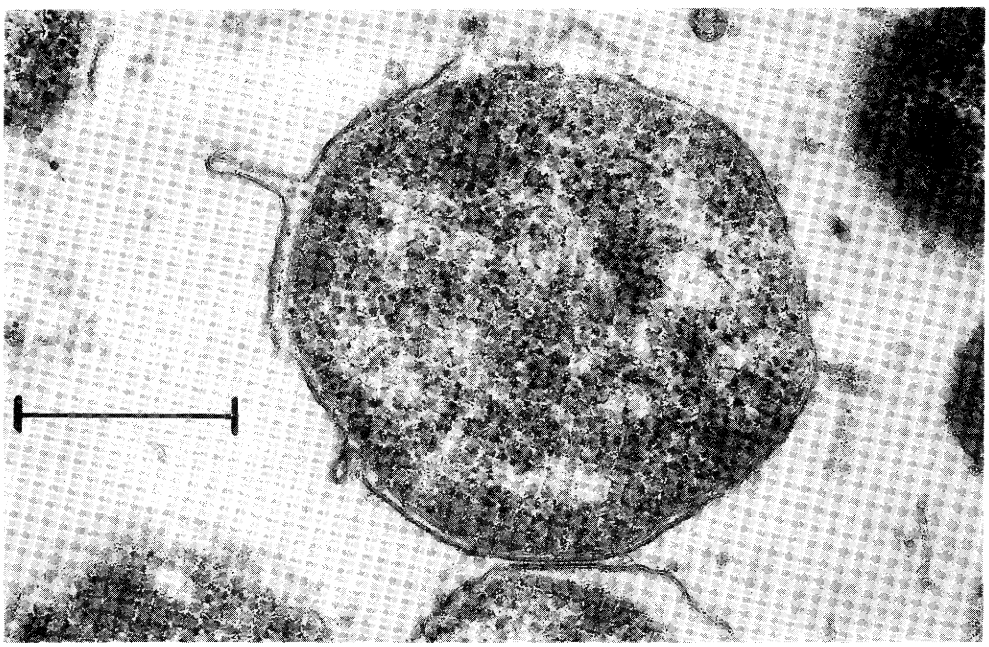


Photo. 2 (Strain K-25)

Four hours culture in the media for inducing pleomorphism. Almost all cells are spherical. Scale indicates 1.0 micron.



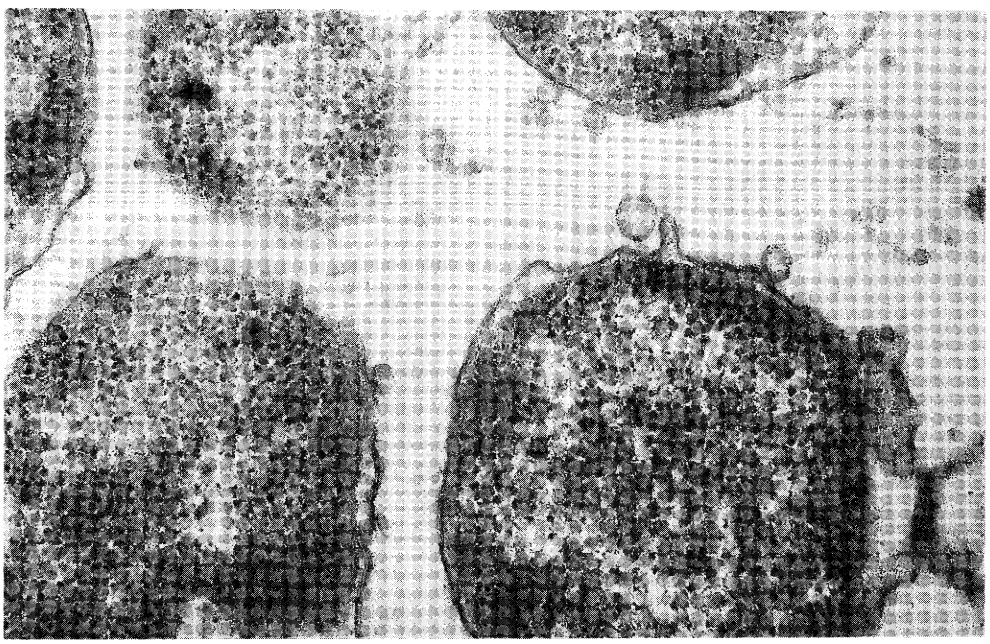


Photo. 3 (upper, Strain 569B), Photo. 4 (lower, Strain K-25).

Note the small protrusions of the cell wall and membrane enveloped particles. Scale indicates 0.5 micron.

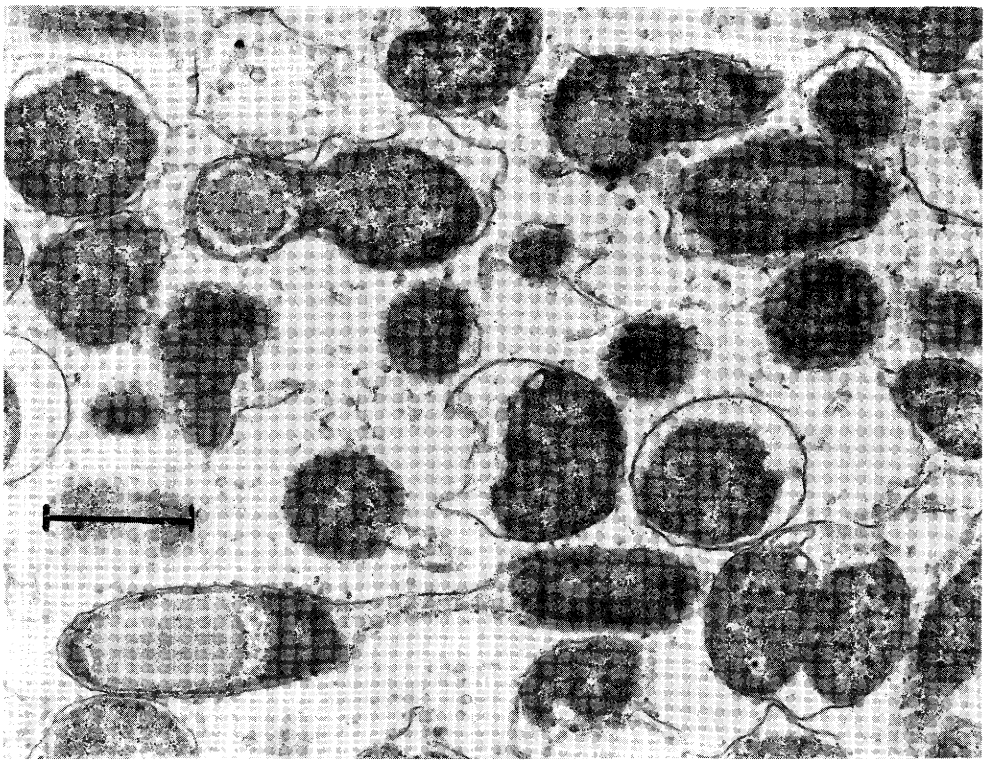


Photo. 5 (Strain 569B).

Crumb-like materials in the cytoplasm are often seen.
Scale indicates 1.0 micron.

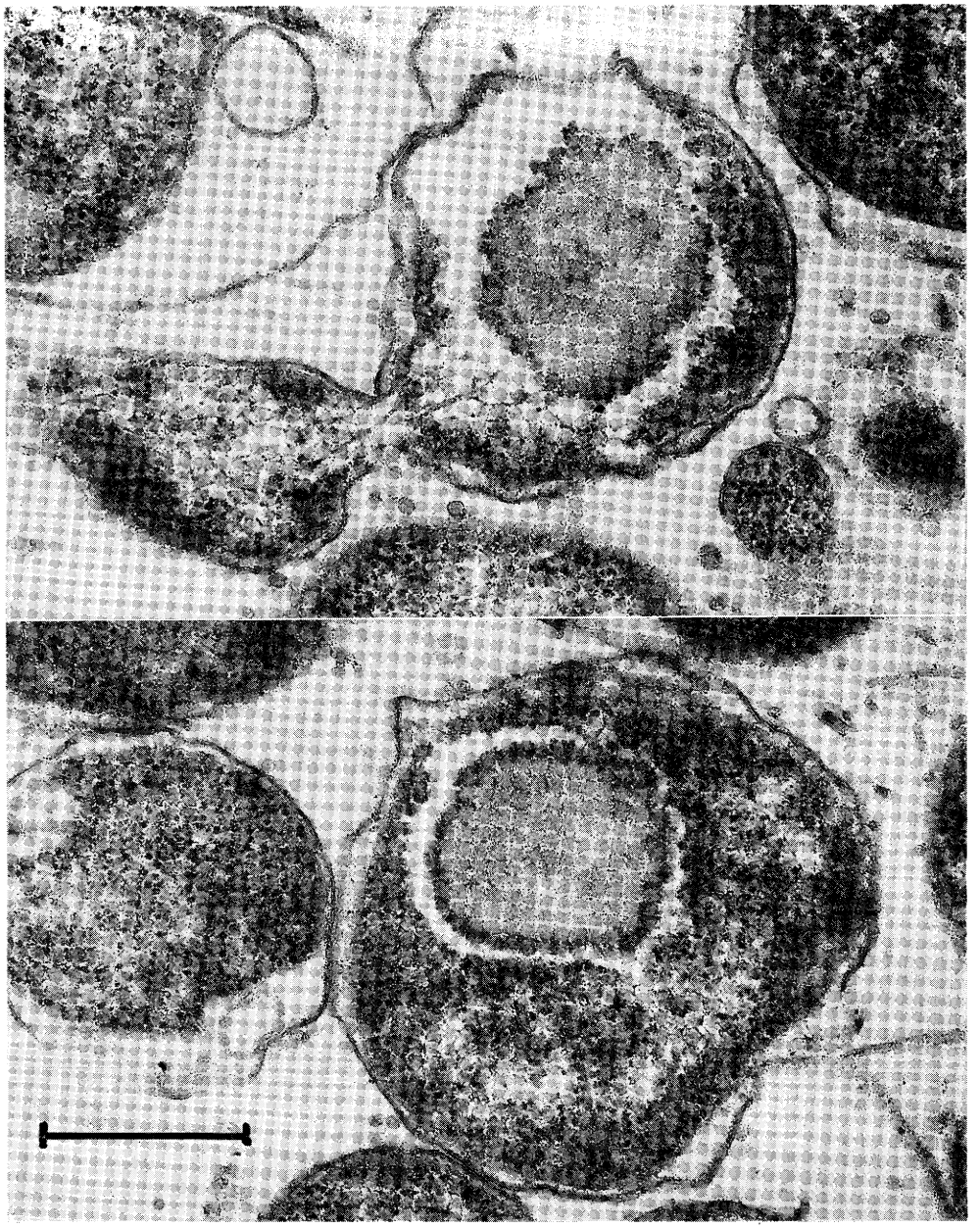


Photo. 6 (Higher magnification of the crumb-like materials, Strain 569B). They are surrounded by ribosome-like particles. Fine net work is connected to the particles. Scale indicates 0.5 micron.

DISCUSSION

The morphological changes of *V. cholerae* during toxin production has been studied by several workers as mentioned previously. In our last report (1979), we speculated that large round cells and membrane enveloped particles, possibly developing from the bulging out and pinching off of the cell wall, played an important role in the release of toxin. But in the present study, *V. cholerae* did not release toxin even though it exhibited similar morphological changes to those seen in the previous study. The proportion of large round cells and membrane enveloped particles to the total number of the cells in this condition was much larger than in the conditions of the previous study (shaking at 30°C, syncase medium). Nevertheless the amount of toxin in the medium was negligible even when *V. cholerae* strain 569B was cultured. The toxin was also not detected in the medium in which *V. cholerae* strain K-25 showed marked morphological changes as mentioned above. These results indicate that this kind of morphological change does not always accompany toxin excretion. In order to clarify the mechanism of toxin secretion, the location of toxin in the cells should be examined. The localization of cholera toxin subunits in fractions of *V. cholerae* was studied by Ohtomo *et al.* (1977). They reported that subunit A was synthesized in the cytoplasm while subunit B was synthesized at the cell membrane or nearby, and suggested that subunit A and B would be assembled into a whole toxin molecule during secretion from the cell. Additional studies are needed to determine the location of toxin in intact cells.

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多形性誘導培地におけるコレラ菌の毒素産生について

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ブイヨンに白糖，硫酸マグネシウム，グリシンをそれぞれ10%，0.2%，1%に加えてコレラ菌を37°C で培養すると，速やかに多形性となり，本来の桿状姿は殆どみられなくなった．この形態はコレラ菌569B株を Syncase medium で30°C 振盪培養した時のものと同様であったが，Syncase medium でみられたような毒素産生は全くみられなかった．また Syncase medium では多形性を示さなかったコレラ菌 K−25株もこの培地では多形性を示したが，毒素の産生はみられなかった．従って，コレラ菌の毒素産生は必ずしも多形性と一致するものではないといえる．

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