FINE STRUCTURE OF Corynebacterium Acnes*

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ABSTRACT

An electron microscopic study was made of $C.\text{acnes}$ grown in vitro and of uninfamed open comedones in acne patients. Seven different strains grown anaerobically for 2–7 days, and comedones were fixed with the Ryter-Kellenberger procedure and cut into thin sections for electron microscopy. Flocular material attached to the cell wall, prominent mesosomes and more simple invaginations of the plasma membrane were characteristic features. The results showed $C.\text{acnes}$ to be similar to other corynebacteria studied previously with the electron microscope and to the organisms observed in large masses within comedones.

*Corynebacterium acnes* is one of the predominant bacteria in normal human skin (1). Renewed interest in this organism has resulted in studies to determine if it plays a role in the pathogenesis of acne vulgaris. $C.\text{acnes}$ has been isolated from comedones and other types of acne lesions (2–6) and the biochemical characteristics of this bacterium have been defined (7, 8). Injection of $C.\text{acnes}$ into steatocystomas resulted in inflammation and rupture of the cysts (9). Similar changes were produced by injection of living, but not dead, $C.\text{acnes}$ into keratinous cysts (10).

Immunologic techniques have revealed that serum antibody levels to $C.\text{acnes}$ are higher in persons with acne than in other individuals, and that the antibody levels parallel the severity of acne (3). It has also been shown that patients with acne have increased serum levels of complement fixing antibodies to $C.\text{acnes}$ (11) and a marked dermal hypersensitivity to the organism (12).

Recently, Zierdt and Wertlake (13) reviewed studies describing $C.\text{acnes}$ as a disease agent in a number of extracutaneous infections. They also examined cultures of $C.\text{acnes}$ with the electron microscope but did not describe its ultrastructure. Thus, we have studied the fine structure of cultured cells of $C.\text{acnes}$ to provide a basis for electron microscopic observation of the host-bacterium relationship in comedones and other acne lesions where additional types of microorganisms may occur (2, 6).

This study was supported by Career Development Award (LFM) 7-K3-AI-31, 210-03, by PHS grant DE 02110, and by equipment grants from The Kresge Foundation and National Science Foundation (GB-7489).

The technical assistance of Miss Gwen Ramer and Miss A. J. Narkates is gratefully acknowledged.

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Received August 28, 1969; accepted for publication November 24, 1969.

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![Fig. 1. Cell of $C.\text{acnes}$ showing cell wall (cw), plasma membrane (pm) and an invagination of the plasma membrane (i). $\times$ 90,000.](image-url)
Fig. 2. Cell of *C. acnes* displaying a large central mesosome (m) and a closely related volutin granule (v). × 132,000.
Fig. 3. Dividing cell of *C. acnes* which has remained attached to another cell (left side of picture) after a previous division. A large central mesosome (m) is shown in the area where septum formation is taking place. Cell wall (cw) and plasma membrane (pm) are beginning to invaginate on opposite sides of the cell. Floccular material (f) adheres to the external surface of the cell. × 85,000.
MATERIALS AND METHODS

Observations were made on 7 strains of *C. acnes*. Four strains were provided by Dr. S. M. Puhvel (University of California, Los Angeles), one strain (6919) was obtained from the American Type Culture Collection, and two strains were isolated from two of our patients. Cultures were grown in the medium of Evans et al. (1) or in fluid thioglycollate medium (Baltimore Biological Laboratories) using an anaerobic jar with a Gaspak envelope. After 48 hours to 7 days of growth, cells were harvested by centrifugation and fixed by the procedure of Ryter-Kellenberger (14). Specimens were dehydrated in acetone and embedded in Araldite (15). Several uninflamed comedones were removed with a comedo extractor from the back and face of the two patients and fixed as described for the cultures. Thin sections of the plastic embedded cultures and comedones were cut on an ultramicrotome and placed on copper grids coated with a carbonized collodion supporting film. Sections stained with lead citrate (16) or without lead staining were reviewed with a Philips EM 200 or a Philips EM 300 electron microscope at 40-100 kv.

RESULTS

Appearance of *C. acnes* in Cultures

Although the cells of each strain differed in size and shape, they possessed many ultrastructural similarities. In sections, bacteria were rounded, oval or elongated. Rounded cells were 0.3-0.6 μ in diameter. The longest cells were 3-4 μ in length and about 0.5 μ in width. Cells from strain ATCC 6919 were strikingly more rounded than cells from the other strains. Cells of each strain showed floccular material adherent to the cell surface and beneath this material was the cell wall. In most cells these two envelopes had a similar electron density and were so closely apposed that they could not be easily differentiated from each other (Figs. 1-3).

The plasma membrane was in contact with the cell wall. It was somewhat wavy and composed of two electron dense layers separated by an electron lucent layer (Figs. 1-7). Mesosomes, produced by whorled invaginations of the plasma membrane, were prominent and clearly showed the trilaminar characteristic of the plasma membrane. Mesosomes lay near and sometimes within the nucleoplasm or were centrally located and related to the septum of dividing cells (Figs. 2, 3, 7). In addition to mesosomes, more simple intracytoplasmic invaginations of the plasma membrane were frequently seen (Fig. 1).

The cytoplasm of *C. acnes* was dense and amorphous. Ribosomes were difficult to detect in most cells because of the dense cytoplasm, but they were sometimes visible in sections stained with lead citrate (Fig. 6). Areas of low electron density, possibly containing lipid, were present in the cytoplasm. In most cells, polyphosphate (volutin) granules were not observed but some were occasionally seen in close relation to mesosomes (Fig. 2).

The nuclear region was usually fairly well circumscribed in one area of the cell.
Fig. 5. These dividing cells of *C. acnes* display different stages of septum formation. Cell division is occurring simultaneously in several different planes. Note the dispersed arrangement of the nuclear material (n). × 90,000.

However, in some instances, it was dispersed and its typical confluent structure was seen in many portions of the cell. Nucleoplasm characteristically showed areas of low electron density filled with DNA filaments (Figs. 4, 5, 9).

Cell division was common and daughter cells often remained attached after completion of septum formation (Figs. 3, 9). Dividing cells often displayed several septa indicating the line of division could occur in more than one plane (Fig. 5).

Appearance of Organisms in Comedones

Longitudinal sections through uninflamed comedones (Figs. 8, 10) showed many free
bacteria associated with cornified cells or embedded in an amorphous material of moderate electron density believed to represent sebum. In those areas of the comedones where bacteria were present in large numbers, the ultrastructural similarities of the organisms were impressive, suggesting the presence of one species. The resemblance of these bacteria to cultured cells of \textit{C. acnes} was also impressive. Examples of these similarities are illustrated in Figures 7-10.

**DISCUSSION**

Zierdt and Wertlake (13) described three different morphologic types in cells of \textit{C. acnes}: a spherical form, a pleomorphic type, and a corynebacterium form which they called the definitive \textit{C. acnes}. Although our study was not designed to confirm that three types exist, some evidence was obtained to support this view. This possibility will be important to consider when studying acne lesions which may include cocci in addition to \textit{C. acnes}.

The floccular material attached to the cell wall was similar to that observed in \textit{C. minutissimum} (17) and \textit{C. ovis} (18). In \textit{C. ovis} (18) the adherence of daughter cells following division was attributed to the cohesive properties of the superficial floccular material. Hard (18) showed that this material is a lipid and Jolly (19) suggested that it may be associated with pathogenicity. In some of our previous studies of \textit{C. minutissimum}, a material of similar appearance was more abundant in \textit{vivo} (erythrasma) than in cultured cells. Electron micrographs (17) depicted the floccular material as contributing to the adherence of diphtheroids to the skin. It remains to be seen if this is also the case with \textit{C. acnes} in human skin.

Prominent mesosomes, such as those seen in this study, have been observed previously in bacteria present in comedones (20). Mesosomes in \textit{B. subtilis} and \textit{B. licheniformis} have been associated with septation and wall-forming capacity. L forms of these organisms, which lack mesosomes, are unable to septate and make cell walls. In contrast, their bacillary forms contain mesosomes and possess this capacity (21). The possibility of the spherical type of \textit{C. acnes} representing an L form was discussed by Zierdt and Wertlake (13). L forms were not seen in our cultures but it will be important to determine whether these occur in skin.

The infrequency of polyphosphate granules seemed to characterize six of the seven strains. It should be remembered, however, that in certain other bacteria (22), including \textit{C. diphtheriae} (23), the polyphosphate content is a function of the growth phase, being lowest in the exponential phase and highest in older cultures. More strains need to be studied before the ability of \textit{C. acnes} to produce polyphosphate granules can be ascertained. Polyphosphate granules are very prominent in \textit{C. minutissimum} (17), the only other member of the cutaneous flora studied \textit{in vitro} and \textit{in vivo} thus far with the electron microscope.

Results of this investigation have revealed striking similarities between many of the bacteria found in uninfamed comedones and cells of \textit{C. acnes} grown \textit{in vitro}. It is noteworthy that a specific immunofluorescent technique has shown a larger number of \textit{C. acnes} in uninfamed comedones (24). Although ultrastructural criteria for recognition of \textit{C. acnes} cannot yet be definitely established, it
Figs. 7-10. Comparison of two cells of cultured C. acnes (Figs. 7 & 9) with two bacteria in comedones (Figs. 8 & 10). Cell wall and plasma membrane are somewhat better outlined in bacteria of comedones (Figs. 8 & 10), but the resemblance of all the cells is striking. × 65,000 (Fig. 7); × 82,000 (Fig. 8); × 62,000 (Fig. 9); × 74,000 (Fig. 10).
seems certain that knowledge gained from this study will provide a useful background for in vivo identification and future observations on the relationship of this bacterium to normal and pathologic skin.

REFERENCES


