Ultrastructural Study of the Host-Bacterium Relationship in Erythrasma

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Erythrasma, for many years considered a superficial fungal infection, has recently been recognized as a bacterial disease. This was first suggested in 1960 by Lagana (Acta Microbiol. Hellen. 5:69, 1960) following isolation of a diphtheroid from lesions of erythrasma. Sarkany, Taplin, and Blank (J. Invest. Dermatol. 37:283, 1961) described the consistent isolation of a gram-positive diphtheroid from lesions of erythrasma. This observation, subsequently confirmed by others (Munro-Ashman, Wells, and Clayton, Brit. J. Dermatol. 75:401, 1963), led to the biochemical characterization of this diphtheroid, Corynebacterium minutissimum, the type strain of which was recently accepted by the National Collection of Type Cultures, London, England (Sarkany, Taplin, and Blank, Lancet 2:304, 1962).

The localization by Sarkany et al. of this organism within cornified cells suggested to us that skin from erythrasma could be used for a study of a human host-bacterium relationship at the ultrastructural level. Also, the frequency of erythrasma, and the accessibility of the lesions to multiple and innocuous biopsies, seemed to offer a good possibility of success for an investigation of this nature.

The 10 adult patients (5 men and 5 women) used in this study fulfilled the diagnostic criteria for erythrasma (clinical appearance, red fluorescence under Wood's light, positive cultures for C. minutissimum, clinical and bacteriological response to systemic erythromycin). Negative mycological studies revealed that these subjects had no superimposed fungal infection on the
patches of erythrasma. The isolation medium described by Sarkany et al. (Arch. Dermatol. 83:578, 1962) was employed for the bacteriological cultures.

Several skin specimens, 2 mm in diameter, were obtained in a manner previously described (Montes, Owens, and Knox, Experientia 20:672, 1964) from the axilla or the crural region of each patient. Immediately after removal, the specimens were fixed according to the Ryter-Kellenberger technique (Z. Naturforsch. 13b:597, 1958). Embedding was performed in a mixture of Epon and Araldite (Mollenhauer, Stain Technol. 39:111, 1964). A Porter-Blum ultramicrotome equipped with a diamond knife was used to prepare the sections. These were cut perpendicularly to the skin surface. An RCA EMU3F electron microscope with an accelerating voltage of 50 kv was utilized for study of the material. From each patient, one specimen was fixed in formalin and embedded in paraffin; 4-μ sections were stained with hematoxylin and eosin, periodic acid-Schiff, and the MacCallum-Goodpasture stain for bacteria in tissue (Mallory, Pathological Technique, W. B. Saunders Co., Philadelphia, 1942). These sections were used for histological observations (Fig. 1-7).

Under the electron microscope numerous bacteria were seen at different levels of the stratum corneum: proliferating over the skin surface (Fig. 3); lying freely between the superficial cornified cells (Fig. 2); penetrating these cells...
from the intercellular spaces (Fig. 4) or, less frequently, directly from the skin surface (Fig. 3); and intracellularly within the keratinized cells (Fig. 2, 5, 6). Whereas most organisms observed on the skin surface were characterized by a homogeneous fine structure, bacteria within the stratum corneum were quite pleomorphic. Furthermore, dividing organisms were more common on the surface than within the stratum corneum.

The stratum corneum itself was hyperkeratotic, with the superficial layers widely separated and the cell boundaries disrupted at the sites of bacterial penetration (Fig. 4). In the keratinized cells, cytoplasmic areas of decreased electron density, frequently observed around intracellular bacteria, were suggestive of a keratolytic process (Fig. 5). Hopefully, studies presently in progress will help to interpret the precise nature of this change. Electron micrographs of the full stratum corneum thickness have shown that bacteria in erythrasma can penetrate as deeply as one-half the thickness of that layer. This observation was confirmed under the light microscope by a study of sections stained with the MacCallum-Goodpasture method for bacteria in tissue. Observation of sections stained with periodic acid-Schiff showed no fungal elements in the stratum corneum of these patients.

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