enzyme preparations, reaction rate with ADP was half of that with ATP, whereas, after 5 min heat treatment at 60°C, activity towards ATP dropped considerably (87%) while that with ADP remained unaffected. Thus there are at least two enzymes responsible for ATP and ADP hydrolysis. The presence of extremely active pyrophosphatase activity having similar pH optima and susceptibility to heat treatment as that of ATP hydrolysing enzyme activity precludes any speculation about the nature of ATP hydrolysis.

The occurrence of F-1,6-di-phosphatase activity in mycobacteria is of interest because of its known involvement in hexose monophosphate pathway[1,2] and in reversal of glycolysis[8]. Both operation of hexose monophosphate pathway[9] and glycolysis reversal – as judged by the formation of glycogen and other polysaccharides together with the presence of other glycolytic enzymes in this organism grown on glycerol as carbon source[2] – are known in mycobacteria. Further, for the interconversion of F-6-P to F-1,6-diphosphate, two different enzymes catalysing the unidirectional reactions are involved. The presence of phosphofructokinase in mycobacteria was reported by GOLDMAN[2], and the present demonstration of F-1,6-diphosphatase activity suggests that a similar diversion of metabolic route occurs here also as in animals[8,10,11].

Zusammenfassung. Die Phosphatase(*n*)-Aktivität der zellfreien Extrakte von *Mycobacterium* 607 wurde untersucht und Pyrophosphat, ATP, ADP und Fructose-1,6-diphosphat hydrolisiert. Eigene Eigenschaften der Phosphatasen wurden studiert. Auch wurde nachgewiesen, dass die Hydrolyse von ATP und ADP durch mindestens zwei verschiedene Enzyme hervorzurufen ist.

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Electron Microscopic Observation of Bacteria Growing on the Skin Surface

The fixation of specimens of human skin containing bacteria, using in our laboratory conventional methods for mammalian tissues (osmium tetroxide, glutaraldehyde, potassium permanganate) failed to result in adequate bacterial fixation. Therefore we decided to try the RYTER-KELLENBERGER (R-K) fixation technique[1].

The areas of the skin to be biopsied were known to possess bacteria because positive cultures of various organisms were obtained either by swabbing or scraping the skin surface. A small punch 2 mm in diameter was used following local infiltration with 2% xylocaine. Immediately after removal, each specimen was immersed in

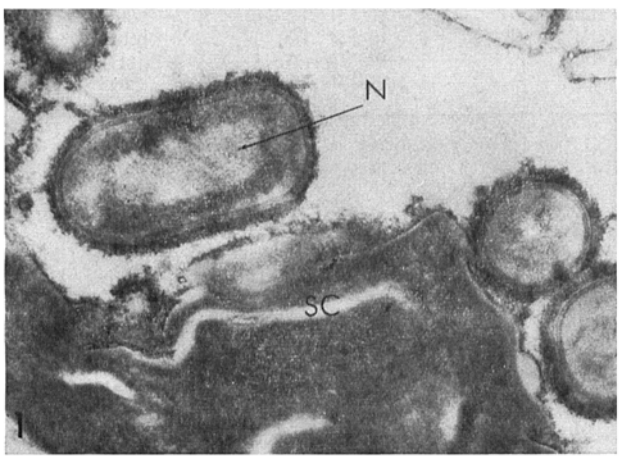

Fig. 1. Bacteria proliferating on the skin surface. The nucleoplasm of one of them (N) and the superficial stratum corneum (SC) are shown (× 36,000).

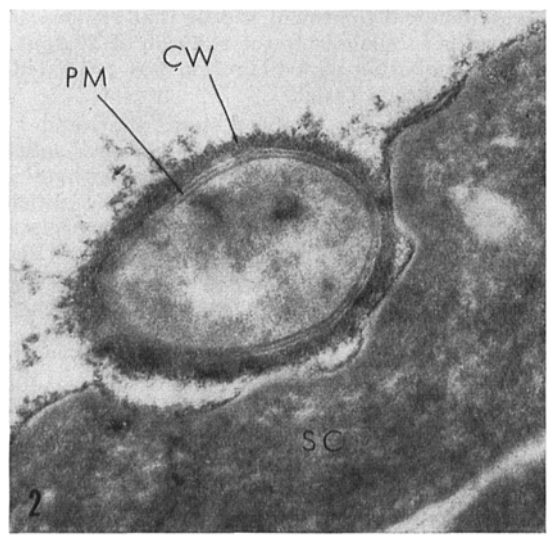

Fig. 2. Bacterium observed right above the stratum corneum (SC). The cell wall (CW) and a double-layered plasma membrane (PM) are seen (× 52,000).

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the R-K fixative and cut in smaller pieces, about 0.25 cm^3. The prefixation step as suggested in the original method was eliminated. Otherwise, the main steps of the procedure were carefully followed. Embedding was performed using a mixture of Epon and Araldite. Observations were performed on an RCA EMU3F electron microscope.

Ten specimens studied so far have included normal skin, skin from individuals with intertrigo (moist areas where skin surfaces are in close contact[2]), and skin from patients with erythrasma (a superficial bacterial infection of th eskin caused by diphtheroids[3]).

Study of this material revealed that bacteria proliferating on the skin surface were adequately fixed. The cell wall and plasma membrane were clearly differentiated (Figures 2, 3). Specializations of the latter forming mesosomes were detected (Figure 3). Electron dense particles were distinguished (Figure 3). Nucleoplasms were also seen (Figure 1). Furthermore, different features of cell division could be observed. Because of the prolonged duration of the fixation, the epidermis itself showed signs of overfixation.

Use of the R-K technique in the way described here should have an interesting application in skin microbiology. It is hoped that both clinical and experimental skin infections will soon be studied at the ultrastructural level. A combination of the R-K method and O'BRIEN's[4] technique for skin inoculation may well provide the ideal tool for this type of study.

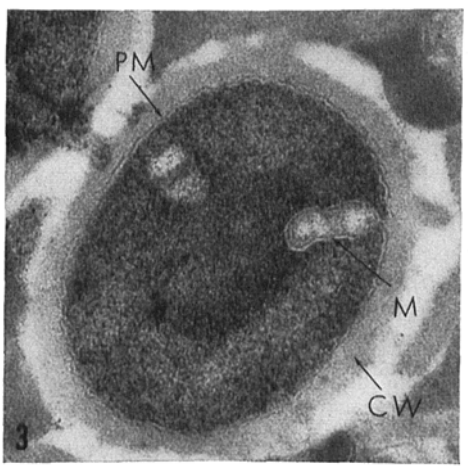

Fig. 3. Bacterium observed on the skin surface. The cell wall (CW) is less dense than the cytoplasm which contains numerous electron dense particles. A mesosome (M) is shown (× 63,000).

Résumé. Des observations au microscope électronique ont été faites sur des bacteries se développant à la surface de la peau. En utilisant la technique de fixation de RYTER-KELLENBERGER, les auteurs ont obtenu bonne préservation des microorganismes, ce qui a permis l'étude de leur fine structure.

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Inhibition of *in vitro* Release of Thyreotrophin by an Analogue of Oxytocin, 3-Valine-oxytocin

In previous experiments[1] we have found evidence for a correlation between adenohypophyseal acid phosphatase activity and the secretion of thyreotrophin (TSH). It was also shown that the non-protein fractions of rat, rabbit and bovine hypothalamic extracts contain a factor activating adenohypophyseal acid phosphatases. The working hypothesis that this factor is identical with the thyreotrophin-releasing factor (TRF) was supported by a large body of indirect evidence[1] and was also verified more directly by the finding that the phosphatase-activating fractions exhibited TRF activity both *in vitro*[2,3] and *in vivo*[4,5]. These fractions, obtained by deproteination of the crude acid aqueous extract of bovine hypothalami followed by paper electrophoresis and gel filtration on Sephadex, contain peptide material yielding the amino acids Asp, Glu, Gly, Ile, Leu, Ser, Thr and Val after acid hydrolysis. As this amino-acid composition somewhat resembles that of oxytocin, and as several synthetic analogues of oxytocin were made available to us[6,7], we considered it worth while to examine the effect of such oxytocin analogues on the release of TSH from rat adenohypophyses *in vitro*.

Essentially, the short-term incubation technique described previously[2,3] was used. Adenohypophyses of male

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