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.

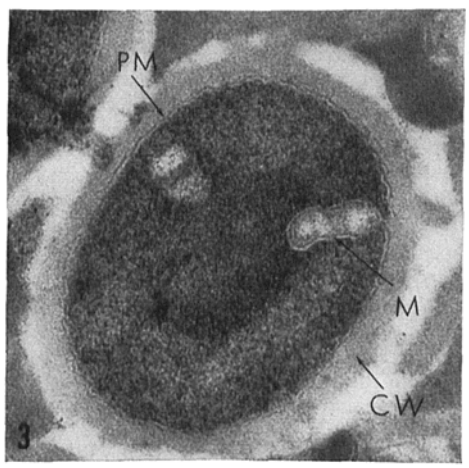

Fig. 3. Bacterium observed on the skin surface. The cell wall (CW) is less dense than the cytoplasm which contains numerous electron dense particles. A mesosome (M) is shown (× 63,000).

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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[5] Supported in part by grants from the Eli Lilly Co., Indianapolis, Indiana and the Upjohn Co., Kalamazoo, Michigan. – We are indebted to Drs. S. H. BLACK, E. KELLENBERGER and R. P. WILLIAMS for their valuable suggestions. – We are also grateful to Miss S. MARTIN, Miss N. MORELAND and Mr. G. ADAMS for their technical assistance.

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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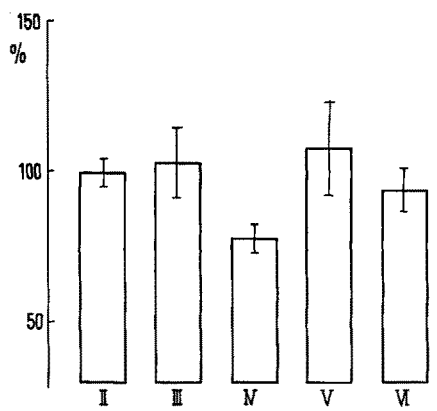
Group	I Controls	II 'Adenohypophyses alone'	III Oxytocin	IV Val³-oxytocin	V Nva³-oxytocin	VI Leu-Gly-Gly-oxytocin
Number of tests	24	23	10	18	5	5
Absolute values (% of radioiodine accumulated in the thyroid gland) Means ± σ_M	11.8 ± 1.10	26.5 ± 0.87	18.3 ± 1.33	20.5 ± 1.9[a]	28.8 ± 4.3	25.1 ± 1.99
Activity in % of controls (Group I = 100%)	100	221 ± 12	205 ± 22	173 ± 15[b]	327 ± 49	170 ± 13
Activity in % of 'adenohypophyses alone' (Group II = 100%)		100 ± 4.4	103 ± 12.1	78 ± 5.0[c]	108 ± 16.3	94.4 ± 6.7

Comparison of the means in groups II and IV by Fisher's t-test: [a] $p \sim 0.05$, , [b] $p < 0.02$, [c] $p < 0.01$.

Wistar rats weighing about 150 g were halved, the halves were divided systematically into individual comparable groups and incubated in Krebs-Ringer bicarbonate medium with 300 mg% glucose at 37°C for 1 h (1 cm³ medium for two half-glands) alone or with the addition of oxytocin, 3-valine-oxytocin, 3-norvaline-oxytocin or N-leucyl-glycyl-glycyl-oxytocin, in concentrations of 50 μg/cm³. The medium was then separated, filtered, and frozen at −15°C. One to three days later it was thawed and injected subcutaneously in amounts of 0.5 cm³ daily for 4 days into female Wistar rats weighing about 100 g and hypophysectomized by the parapharyngeal approach three days before the first injection. Each rat thus received a total dose of 2 cm³, corresponding to four half-glands. 1 h after the last injection the rats received 5 μC of carrier-free $Na^{131}I$ intraperitoneally and 24 h later they were killed by ether anaesthesia. The thyroids were removed and their radioactivity measured by a Geiger-Müller counter, a model of rat thyroid containing the full dose of $Na^{131}I$ being used as reference and the activity of each thyroid being expressed as a percentage of the total dose.

In each of six experiments, group I (control rats without any injection of incubation media) and II (rats given medium after the incubation of adenohypophyses only) together with one or two of the other groups were included in order to obtain an adequate control of the basal level of TSH release in different groups of glands. The effect of the various synthetic peptides (Table) could therefore be assessed not only with reference to the control group I (the accumulation of radioiodine in the thyroids of this group being taken as 100%), but also as a percentage of the TSH activity of the corresponding 'adenohypophyses only' incubates (group II). Because of the variation in the level of TSH release by the control adenohypophyses ('adenohypophyses only', group II) in different experiments the latter method is to be preferred (Figure). Thus the low level of TSH activity in the medium after incubation with synthetic oxytocin was due to a low TSH release by the adenohypophyses used in the experiment as a whole: the apparent effect vanishes when the activity in group III (oxytocin) is expressed as a percentage of the TSH release in group II ('adenohypophyses only') of the same experiment.



TSH activity in incubation media of groups II (adenohypophyses alone), III (oxytocin), IV (Val³-oxytocin), V (Nva³-oxytocin), and VI (Leu-Gly-Gly-oxytocin). Radioactivity of the thyroids of rats receiving the medium after incubation of the 'adenohypophyses alone' was taken as 100%.

Of the four peptides tested, only one, 3-valine-oxytocin, exhibited a distinct effect in this system, causing a significant inhibition of TSH release from the adenohypophyses *in vitro*. As yet this result is difficult to interpret. Attempts to demonstrate inhibition of TSH release *in vivo*, measuring the release of radioiodine pre-accumulated in the thyroid gland of rats after intravenous injection of 3-valine-oxytocin, have so far failed to give consistent results. Preliminary experiments indicate that 3-valine-oxytocin blocks the activating effect of hypothalamic extracts on adenohypophyseal acid phosphatases *in vitro*; the possibility that the peptide acts as an antagonist of the TRF is being examined[8].

Zusammenfassung. Rattenadenohypophysen wurden in Krebs-Ringer-Bikarbonatlösung ohne Zusatz oder mit 50 μg/cm³ synthetischem Oxytocin, 3-Valin-Oxytocin, 3-Norvalin-Oxytocin und Leucyl-Glycyl-Glycyl-Oxytocin inkubiert. Die Freisetzung von TSH wurde durch 3-Valin-Oxytocin gehemmt; die anderen Peptide waren ohne Wirkung.

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