On the basis of uronic acid values, the total amount of glycosaminoglycan in the intimal layers of pig aorta was about 10–12 mg/g of the dry defatted tissue. The approximate proportions of the component glycosaminoglycans were: hyaluronic acid, 8%; heparan sulphate, 15%; chondroitin sulphate, 65%; dermatan sulphate, 4%. The total amount agreed with KAPLAN and MEYER's values[11] for human aorta, but was somewhat less than values reported by other authors who have used human, guinea-pig and rabbit material[12–15]. The low value for dermatan sulphate and the corresponding high chondroitin sulphate value appear to be characteristic of young aortic tissue[11], although the absolute value for the dermatan sulphate was lower than expected[16]. The hyaluronic acid value was also lower than those previously reported for tissues of similar age from other species[11–15,17].

Zusammenfassung. Mittels einer modifizierten Fällungsmethode mit Cetylpyridiniumchlorid wurde die Verteilung der Glykosaminoglykanen in den innern Schichten junger Schweineaorten festgestellt. Typisch für junges Aortengewebe waren niedrige Dermatansulfat- und entsprechend hohe Chondroitinsulfatwerte. Dermatansulfat- und Hyaluronsäurewerte blieben niedriger als bei bisher beschriebenem Aortengewebe anderer Tierarten in vergleichbarem Alter.

J. R. DUNSTONE

Department of Physical Biochemistry, John Curtin School of Medical Research, Australian National University, Canberra (Australia), 7th November 1966.

[11] D. KAPLAN and K. MEYER, Proc. Soc. exp. Biol. Med. *105*, 78 (1960).
[12] G. S. BERENSON, Circulation Res. *7*, 889 (1958).
[13] I. GORE, Y. TANAKA, T. FUJINAMI and M. L. GOODMAN, J. Nutr. *87*, 311 (1965).
[14] C. J. F. BÖTTCHER and F. B. KLYNSTRA, J. Atheroscler. Res. *2*, 263 (1962).
[15] T. LACSON, D. S. MCCANN and A. J. BOYLE, J. Atheroscler. Res. 6, 277 (1966).
[16] M. D. FRANEK and J. R. DUNSTONE, Biochim. biophys. Acta *127*, 213 (1966).
[17] Acknowledgements: This investigation was supported, in part, by the National Heart Foundation of Australia. The technical assistance of Miss D. CREBBIN is appreciated.

The Fine Structure of *Corynebacterium minutissimum*

Erythrasma, a superficial bacterial infection of the skin, is caused by proliferation of a diphtheroid in the horny layer[1,2]. In view of the relatively little information available on the microanatomy of the corynebacteria[3] and as antecedent to an investigation of the ultrastructure of the horny layer in erythrasma, we have undertaken to study the fine structure of the causative agent, *Corynebacterium minutissimum*, the type strain of which was recently accepted by the National Collection of Type Cultures, London (England)[2].

10 strains originally isolated from patients with erythrasma were used. 8 were supplied by the National Collection of Type Cultures, London (England), another was provided by D. TAPLIN, University of Miami (Florida), and one was isolated from one of our patients. The medium described by SARKANY et al.[4] was used for isolation and maintenance of the organisms. For electron microscopy, they were subcultured in nutrient broth (Difco) and in blood agar, and fixed using the RYTER-KELLENBERGER[5] procedure. As described before, the same fixation technique was used for biopsied skin obtained from lesions of erythrasma[6]. Embedding was performed in a mixture of Epon and Araldite[7], sections were cut with a Porter-Blum microtome equipped with a diamond knife and observed in a RCA EMU3F electron microscope using an accelerating voltage of 50 kv.

C. minutissimum is seen in sectioned cultured cells (Figure 1) as slightly elongated rods surrounded by an electron-dense, surface-adhering material. Underneath this material lies the cell wall, a tripartite structure consisting of 2 bands (35 Å wide) separated by a light zone 50 Å wide. The plasma membrane was only occasionally well resolved in our preparations. Large, irregularly shaped, dense granules (volutin) were a striking feature

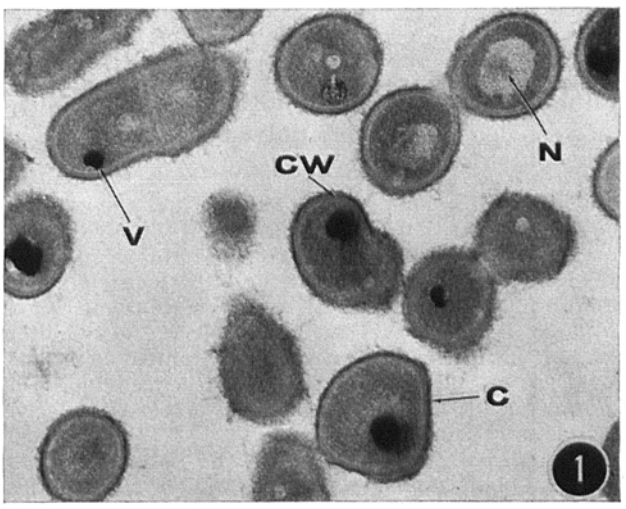

Fig. 1. Thin section through cells of *C. minutissimum*. The cell wall (CW) and the electron-dense surface adhering material (C) are shown. Several volutin granules (V) and nucleoplasms (N) are seen also. Broth culture incubated for 16 h. × 45,900.

[1] I. SARKANY, D. TAPLIN and H. BLANK, J. invest. Derm. *37*, 283 (1961).
[2] I. SARKANY, D. TAPLIN and H. BLANK, Lancet *2*, 304 (1962).
[3] T. KAWATA, Japan. J. Microbiol. *5*, 442 (1961).
[4] D. TAPLIN, I. SARKANY and H. BLANK, A.M.A. Archs Derm. *85*, 578 (1962).
[5] A. RYTER and E. KELLENBERGER, Z. Naturf. *135*, 597 (1958).
[6] L. F. MONTES, D. W. OWENS and J. M. KNOX, J. invest. Dermat. *45*, 93 (1965).
[7] H. MOLLENHAUER, Stain Technol. *39*, 111 (1964).

in the cytoplasm. Ribosomal particles were also observed. Mesosomes were seen in close proximity to, and sometimes continuous with, the plasma membrane. They were often found near the septum in dividing cells.

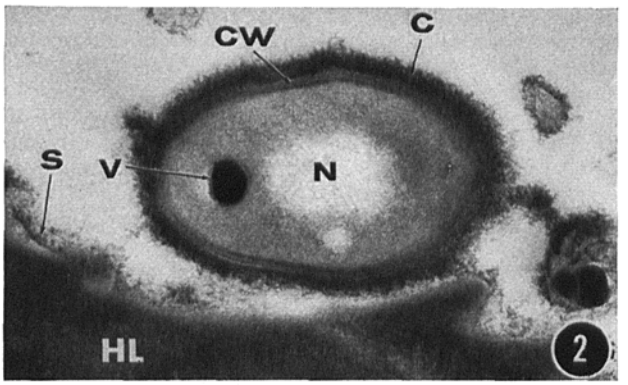

Fig. 2. *C. minutissimum* observed on the skin surface (S) right above the horny layer (HL). Visible in this picture are the surface adhering material (C), the cell wall (CW), the nucleoplasm (N) and a volutin granule (V). Biopsy from a lesion of erythrasma. × 97,200.

These features are apparent also in diphtheroids observed in sections of the erythrasma lesions themselves, particularly at the level of the skin surface (Figure 2)[8,9].

Zusammenfassung. Die charakteristischen Ultrastrukturen vom Erythrasma verursachenden *Corynebacterium minutissimum* waren: 1) dreifache Zellwand, 2) Mesosomen und 3) leicht elektronendurchlässige cytoplasmische Einschlüsse («volutin»). Im Laboratorium kultivierte Zellen und solche aus der Haut von Patienten mit Erythrasma zeigten die gleichen Strukturmerkmale.

L. F. MONTES and S. H. BLACK

Departments of Dermatology and Microbiology, Baylor University College of Medicine, Houston (Texas, USA); 16th September 1966.

[8] This investigation was supported by USPHS Research Career Development Award No. 1-K3-A1-31,210-01 (Dr. MONTES), by a Research Grant from the Eli Lilly and Company, Indianapolis (Indiana) and by a contract (No. DA-49-193-MD-2746) from the U.S. Army Medical Research and Development Command. Office of the Surgeon General.

[9] The technical assistance of NEDRA MORELAND, SYLVIA MCCREVEY and G. ADAMS is gratefully acknowledged.

Solubility of Cobalt Laurate in Water and Non-Aqueous Solvents

In contrast to other heavy metal soaps, the cobalt soaps almost insoluble both in aqueous and non-aqueous media find sparse industrial applications[1,2]. It was, therefore, thought worthwhile to determine the solubility of cobalt laurate in various solvents and to devise mixtures of nonaqueous solvents which may dissolve cobalt soaps in large excess. Usual methods[3-10] viz., evaporation method and chemical analysis of saturated solutions, could not be employed. The radio tracer technique well known for its high sensitivity was expected to give satisfactory results.

Experimental. Preparation of the labelled cobalt laurate. Cobalt-58 in the form of $CoCl_2$ in HCl solution was obtained from the Isotope Division, Atomic Energy Establishment, Bombay. Cobalt laurate was precipitated by the addition of a mixture of ordinary reagent $CoCl_2$ with Co^{58} enriched $CoCl_2$ sample to stoichiometric amount of Na-laurate (prepared from reagent grade NaOH and lauric acid) in water, washed with distilled water and then with alcohol to remove free precipitant, dried in vacuum dessiccator and stored in stoppered bottle. The activity labelled was about 1.2 ± 0.12 millicurie/g of Co-laurate.

Saturated solutions of Co-laurate were prepared by agitation of an excess of Co-laurate in large Pyrex stoppered bottles about $^1/_2$ full of the solvent till true solubility equilibrium was established. Attainment of this equilibrium was indicated by constancy of the counting rate (activity) on successive leaching cycles, independent of time of additional agitation and the amount of excess solid phase present.

Analysis. Samples withdrawn at various intervals were filtered through a fine paper, 10 ml were taken for counting purposes. Specific activity of Co-laurate was determined in terms of count rate by preparing its standard solution in 1:1 benzene/methanol mixture. γ-ray scintillation spectrometer (Atomic Energy Establishment, Bombay) employing NaI (Tl) crystal was used for activity measurements. All precautions were taken to ensure that activity measurements with saturated solutions of Co-laurate in various solvents and its standard solution were made under the same geometrical conditions. The spectrometer was calibrated with various γ-ray energies. The counting rate was taken using it as integral spectrometer keeping the bias at 3 volts and also at the photopeak of the γ-ray of 0.81 MeV. The solubility values calculated both ways were the same, within statistical fluctuations. Appropriate corrections were made for coincidence loss and the decay of the isotope.

Results. The solubility values with probable statistical errors are given in the Table. It is evident that Co-laurate is very sparingly soluble both in aqueous and

[1] A. S. C. LAWRANCE, J. Instn Petrol. Technol. *24*, 207 (1938).
[2] A. S. C. LAWRANCE, J. Instn Petrol. Technol. *31*, 202 (1945).
[3] J. ZINK and R. LIERE, Z. angew. Chem. *28*, 229 (1915).
[4] W. FAHRION, Chem. Umschau Geb. FeHe *23*, 34 (1916); J. Soc chem. Ind., London *35*, 932 (1916).
[5] W. LANGLEY, M. G. ROSENBAUM and M. M. ROSENBAUM, J. biol. Chem. *99*, 271 (1932).
[6] B. H. KEMP and F. H. FISH, Virginia J. Sci. *1*, 127 (1940).
[7] N. P. DATTA, J. Indian chem. Soc. *16*, 573 (1939).
[8] A. DOBRY, J. phys. Chem. Ithaca *58*, 576 (1954).
[9] S. G. DANIEL, Trans. Faraday Soc. *47*, 1351 (1951).
[10] C. A. JACOBSON and A. HOLMES, J. biol. Chem. *25*, 29 (1916).