

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.

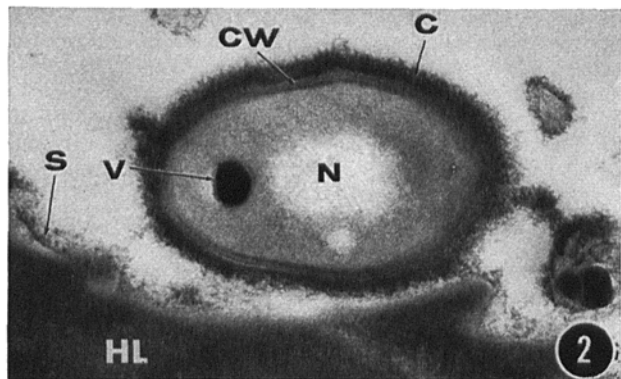


Fig. 2. *C. minutissimum* observed on the skin surface (S) right above the horny layer (HL). Visible in this picture are the surface adhering material (C), the cell wall (CW), the nucleoplasm (N) and a volutin granule (V). Biopsy from a lesion of erythrasma.  $\times 97,200$ .

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)<sup>8,9</sup>.

**Zusammenfassung.** Die charakteristischen Ultrastrukturen vom Erythrasma verursachenden *Corynebacterium minutissimum* waren: 1) dreifache Zellwand, 2) Mesosomen und 3) leicht elektronendurchlässige cytoplasmatische 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.

<sup>8</sup> 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.

<sup>9</sup> 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<sup>1,2</sup>. 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<sup>3-10</sup> 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  $\text{CoCl}_2$  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  $\text{CoCl}_2$  with  $\text{Co}^{58}$  enriched  $\text{CoCl}_2$  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 dessicator and stored in stoppered bottle. The activity labelled was about  $1.2 \pm 0.12$  milli-curie/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  $\frac{1}{2}$  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.  $\gamma$ -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  $\gamma$ -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  $\gamma$ -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

<sup>1</sup> A. S. C. LAWRENCE, J. Instn Petrol. Technol. 24, 207 (1938).

<sup>2</sup> A. S. C. LAWRENCE, J. Instn Petrol. Technol. 37, 202 (1945).

<sup>3</sup> J. ZINK and R. LIERE, Z. angew. Chem. 28, 229 (1915).

<sup>4</sup> W. FAHRION, Chem. Umschau Geb. FeHe 23, 34 (1916); J. Soc. chem. Ind., London 35, 932 (1916).

<sup>5</sup> W. LANGLEY, M. G. ROSENBAUM and M. M. ROSENBAUM, J. biol. Chem. 99, 271 (1932).

<sup>6</sup> B. H. KEMP and F. H. FISH, Virginia J. Sci. 1, 127 (1940).

<sup>7</sup> N. P. DATTA, J. Indian chem. Soc. 16, 573 (1939).

<sup>8</sup> A. DOBRY, J. phys. Chem. Ithaca 58, 576 (1954).

<sup>9</sup> S. G. DANIEL, Trans. Faraday Soc. 47, 1351 (1951).

<sup>10</sup> C. A. JACOBSON and A. HOLMES, J. biol. Chem. 25, 29 (1916).

non-aqueous solvents. However, its solubility in methanol is abnormally high. This high solubility can be attributed to some sort of solute-solvent interaction. Co-laurate, when dissolved in methanol, shifts the equilibrium  $\text{CoL}_2 \rightleftharpoons \text{Co}^{2+} + 2\text{L}$  towards the right due to the removal of  $\text{Co}^{2+}$  ions by methanol resulting in high solubility. Luz<sup>11-13</sup> recently reported the existence of a hexamethanol complex  $[\text{Co}(\text{MeOH})_6]^{2+}$ , in methanol solution of  $\text{Co}^{2+}$  ions containing different amounts of chloride ions, at very low temperatures and the existence of the equilibrium  $[\text{Co}(\text{MeOH})_6]^{2+} \rightleftharpoons [\text{Co}(\text{MeOH})_5\text{Cl}]^+$  at higher temperatures. The relatively higher solubility of Co-laurate

in methanol may be explained in terms of the existence of such cobalt complexes as  $[\text{Co}(\text{MeOH})_6]^{2+}$  and  $[\text{Co}(\text{MeOH})_5\text{L}]^+$  (L = Laurate).

It has been observed that the solubility of cobalt soap in benzene increases abnormally by adding small amounts of methanol. Solubility of Co-laurate in 1:9 methanol-benzene mixture is about 1000 times that of the cobalt soap in benzene.

Further work on the effect of the additives on the solubility of Co-laurate is in progress.

**Zusammenfassung.** Die bisher unbekannte Löslichkeit von Kobaltlaurat wurde mit Hilfe der Tracer-Technik unter Verwendung von  $^{58}\text{Co}$  bestimmt. Die Löslichkeit in Wasser und organischen Lösungsmitteln (mit Ausnahme von Methanol) ist sehr gering. Die relativ grosse Löslichkeit in Methanol wird auf Bildung von  $\text{Co}^{2+}$ -Methanol-Komplexen zurückgeführt.

WAHID U. MALIK, B. P. SINGH and A. K. JAIN

*Chemical Laboratories and Department of Physics, University of Roorkee (U.P., India), 11th October 1966.*

Solubility of cobalt laurate in water and non-aqueous solvents at 30°C.

Solvents	Solubility $\cdot 10^5$ (M/l)
Water	$2.295 \pm 0.094$
Dimethyl formamide	$1.992 \pm 0.022$
Methyl ethyl ketone	$2.121 \pm 0.025$
Acetone	$2.396 \pm 0.025$
Ethanol abs.	$3.943 \pm 0.061$
Methanol abs.	$604.25 \pm 3.01$
Benzene	$7.985 \pm 0.087$
Cyclohexane	$4.435 \pm 0.058$
Toluene	$3.967 \pm 0.074$
Carbon tetrachloride	$1.159 \pm 0.029$

### Adherence of Opsonized Lymphocytes to Macrophage Cultures

Adherence or phagocytosis of opsonized particles by macrophages is well known and has been used to detect antibodies to bacteria<sup>1,2</sup>, erythrocytes<sup>3,4</sup> and rather less commonly nucleated cells<sup>5,6</sup>. Phagocytosis of tumour cells<sup>6</sup> by macrophages has been demonstrated in the presence of immune serum. Since the immune response to a transplantable tumour<sup>7</sup> may be vigorous it seemed worthwhile to determine whether immunophagocytosis might still be useful for the detection of iso-antibody when induced by the injection of normal cells. 2 advantages of this technique are that results can be read easily and the preparations may be stored as permanent records. The sensitivity of immunophagocytosis was compared directly with agglutination<sup>8</sup> and cytotoxic tests<sup>9</sup> for iso-antibodies.

The present experiment utilized cultures of extended macrophages (24 h old), and normal lymphocytes were used for interaction with macrophages in the presence of iso-antibody. An immune serum was prepared by injecting mice with lymphocytes from a different strain. This serum was incubated overnight with tissue culture preparations of normal macrophages. When normal lymphocytes from the donor animals were added to these cells a marked degree of adherence and phagocytosis was observed. Normal serum failed to give any significant reaction. 30 CBA mice (Animal Suppliers, London) were given  $100 \cdot 10^6$  lymphocytes by the i.p. route every 3 days for a total of 6 injections. The lymphocytes were obtained by grinding axillary, inguinal, mesenteric and

submandibular lymph nodes in a hand operated homogenizer with a  $\frac{1}{32}$  inch clearance between the plunger and the barrel. The animals were bled 7 days after the last injection and the serum sterilized by passage through a Millipore Swinnex filter. It was stored at 4°C and used the following day. The cytotoxic test<sup>9</sup> for demonstration of mouse antibody was used to measure the titre of the anti-lymphocytic serum. The lymphocytes were taken from the mesenteric, inguinal, axillary and submaxillary glands of normal C57B1 mice. The lymph nodes were teased into a suspension and injected through a No. 19 needle. The macrophage monolayers were prepared from normal CBA mice<sup>10</sup>. The animals were killed, the abdominal cavity rinsed out with 2 ml of medium 199 (Glaxo) containing 5 units of heparin/ml and the cell count adjusted to  $1 \cdot 10^6/\text{ml}$ . Sufficient normal or immune CBA

<sup>1</sup> G. BIOZZI, C. STIFFEL, L. LE MINOR, D. MOUTON and Y. BOUTHILLIER, *Annls Inst. Pasteur*, Paris 105, 635 (1963).

<sup>2</sup> S. SLOPEK, K. GRZYBEK-HRYNCEWICZ, J. LADOSZ and K. KUBIS, *Archs Immun. Ther. Exp.* 13, 302 (1965).

<sup>3</sup> C.-S. WRIGHT, M. C. DODD, N. G. BRANDT, S. M. ELLIOTT and J. H. BASS, *J. Lab. & clin. Med.* 41, 169 (1953).

<sup>4</sup> A. E. STUART and R. A. CUMMING, *Vox Sang*, in press (1967).

<sup>5</sup> P. MIESCHER, *Dt. med. Wschr.* 83, 216 (1953).

<sup>6</sup> B. BENNET, L. J. OLD and E. A. BOYSE, *Transplantation* 2, 183 (1964).

<sup>7</sup> A. M. EL HASSAN and A. E. STUART, *Br. J. Cancer*, 19, 343 (1965).

<sup>8</sup> D. B. AMOS, *Br. J. exp. Path.* 34, 464 (1953).

<sup>9</sup> E. A. BOYSE, L. J. OLD and I. CHOUROULINKOV, *Meth. med. Res.* 10, 39 (1964).

<sup>10</sup> J. L. WHITBY and D. ROWLEY, *Br. J. exp. Path.* 40, 357 (1959).