

# PRODUCTION OF EXTRACELLULAR TOXIN BY *LISTERIA MONOCYTOGENES* IN VITRO

## Preliminary Report

A. SOUČEK, F. PATOČKA, ANNA SOUČKOVÁ

Department of Medical Microbiology, Charles University, Prague

In recent years, interest has been drawn to toxin production by *Listeria monocytogenes*. Up to now, nobody has succeeded in demonstrating toxin production in vitro (1, 2). Attempts have been made to find virulence factors in endoplasmic material; for instance, Stanley (3) demonstrated a factor stimulating monocytosis and Patočka et al. (4) demonstrated a protein promoting *Listeria* infection on mice. An important advance was the demonstration of changes induced in rabbits in which living *Listeria* organisms were implanted in diffusible chambers; the effect was lethal and examination showed hyperaemia of the liver and splenomegaly (1). The results were indicative of production of a filtrable toxic component. Similar experiments in mice were negative and the authors in question (2) concluded that *Listeria* organisms did not produce toxin in vivo.

Patočka (5) demonstrated that endoplasmic material obtained by mechanical disruption of the bacterial cells produced oedema when administered intradermally to rabbits. For demonstrating toxin in the present experiments we used supernatant of 24-hour broth cultures incubated in Todd-Hewitt broth at 37° and 20°C. After filtering the supernatant through membrane filters and adding merthiolate in the ratio 1 : 5,000, we concentrated it by salting out with ammonium sulphate up to 50% saturation, followed by cold methanol fractionation by the Cohn VI method; we then dissolved the concentrate in saline containing phosphate buffer at pH 7.6. The yield from 1,000 ml. supernatant was 10 ml. purified substance. When this was administered intravenously to a rabbit in 2 ml. volume, the animal died within 12 hours; 0.5 ml. of the sample administered intravenously to a mouse did not produce any changes. When administered intradermally to a rabbit it produced oedema after two hours, followed by central necrosis and haemorrhage. Because of the striking analogy between this dermonecrotic and lethal effect and the effect of the toxin of *Corynebacterium pyogenes* var. *hominis*, *C. pseudotuberculosis* and *C. ulcerans*, we investigated the enzymatic activity of the purified substance (6, 7, 8, 9, 10, 11). It was found that it degraded sphingomyelin to N-acylsphingosylphosphate and choline and that it inhibited

the haemolytic effect of *Clostridium perfringens*  $\alpha$ -toxin. Our experiments provide evidence that *Listeria monocytogenes*, under the given experimental conditions, releases into the medium during growth a toxin with dermonecrotic and lethal activity for rabbits and a phospholipase of the phosphatidylcholine-phosphatidohydrolase group (E.C. 3.1.4.1).

#### REFERENCES

1. **McIlwain, P. K., Barnes, R. W.:** Amer. J. Veter. Research 29, 1968 : 483. — 2. **DiCapua, R. A., Osebold, J. W., Stone, K. R.:** Amer. J. Veter. Research 29, 1968 : 2023. — 3. **Stanley, N. F.:** Austral. J. Exptl. Biol. and Med. Sci. 27, 1949 : 123. — 4. **Patočka, F., Mára, M., Schindler, J.:** Zbl. Bakt. Abt. I. Orig. 174, 1959 : 586. — 5. **Patočka, F.:** unpublished results. — 6. **Patočka, F., Mára, M., Souček, A., Součková, Anna:** J. Hyg. Epidem. Microbiol. Immunol. 6, 1962 : 1. — 7. **Souček, A., Součková, Anna, Mára, M., Patočka, F.:** J. Hyg. Epidem. Microbiol. Immunol. 6, 1962 : 13. — 8. **Souček, A., Michalec, M., Součková, Anna:** Biochim., Biophys. Acta 144, 1967 : 180. — 9. **Souček, A., Součková, Anna, Patočka, F.:** J. Hyg. Epidem. Microbiol. Immunol. 11, 1967 : 123. — 10. **Souček, A., Součková, Anna:** Proc. XIth Conference of Charles Univ. Medical Faculty Prague 1968, p. 70. — 11. **Součková, Anna, Souček, A.:** Proc. XIth Conference of Charles Univ. Medical Faculty Prague 1968, p. 85.

Received April 24, 1969.

A. Souček, Ústav pro lékařskou mikrobiologii,  
Studničkova 7, Praha 2, Czechoslovakia