

antitoxin was detected in broth cultures. In broth culture filtrates of *Corynebacterium ulcerans* strains we demonstrated a toxic protein different from diphtheria toxin, with a characteristic effect in experimental animals. In guinea pigs and rabbits it produced haemorrhagic necrosis similar to the form which develops after the administration of *Corynebacterium pseudotuberculosis* toxin. The toxic protein also inhibited the haemolytic effect of *Staphylococcus pyogenes* alpha and beta lysin and of *Clostridium perfringens* alpha toxin. Study of the behaviour of this toxin on Sephadex and ion exchangers and in electrophoresis showed that it had different mobility from *Corynebacterium pseudotuberculosis* toxin in electrophoresis. Identity of the toxin with an enzyme of the phosphatidylcholine phosphatidohydrolase group with an affinity for sphingomyelin and lysolecithin was demonstrated. The enzyme also decomposed these phospholipids in serum lipoproteins. An attempt to identify this enzyme with the factor which induces turbidity formation in animal sera was unsuccessful.

Correlation of Growth and Cytochrome b₁ Production in Corynebacterium haemolyticum to the Haemin Concentration in the Medium. M. MÁRA, D. KALVODOVÁ, F. PATOČKA, Laboratory for Special Medical Microbiology, Faculty of Medicine, Charles University, Prague; Department of Medical Microbiology and Immunology, Faculty of Medicine, Charles University, Prague.

In a study of the taxonomic classification of *Corynebacterium haemolyticum* we demonstrated cytochrome b₁ with differential spectrum maxima at 430, 530 and 560 nm in the microbial cells. This finding differentiated the microorganisms from pyogenic streptococci, but we failed to demonstrate production of a complete cytochrome system as known in *Corynebacterium diphtheriae*. Later, after adding haemin to the medium, Patočka and Kalvodová observed improved production and the development of L-forms of *Corynebacterium haemolyticum*. With reference to these findings and to the structural similarity of haemin to group b cytochromes, we attempted to determine the effect of haemin on growth and cytochrome b₁ synthesis. It was found that growth of the microorganism depended on the presence of haemin in the medium and that this dependence was more marked under aerobic conditions, when growth in liquid synthetic media and on Tryptic-Soy-Broth agar was minimal. Haemin stimulated growth in a dose range of 1–20 µg/ml medium. Under anaerobic conditions, haemin also improved growth on solid medium in amounts of up to 10 µg/ml medium. Similar dependence was observed when determining cytochrome b₁ in the particulate fraction of the bacterial endoplasm obtained by differential centrifugation of disintegrated bacterial cells. The amount of particulate fraction rose proportionally to the amount of haemin

in the medium within a dose range of 1–20 µg/ml. Cytochrome b₁ was also produced in small amounts under anaerobic cultivation conditions. It was found that haemoglobin could be substituted for haemin, but the effect was smaller. The results show a correlation between growth and cytochrome b₁ production, particularly under aerobic conditions.

Listeria monocytogenes Lipids: Their Dependence on the Cultivation Conditions and Strain Virulence. M. MÁRA, B. BENEŠ, F. PATOČKA, Laboratory for Special Medical Microbiology, Faculty of Medicine, Charles University, Prague.

Renewed attention has recently been evidenced in the lipids of *Listeria* organisms and the relationship of these substances to the virulence of the strain. In this study the authors investigated the total lipid and fatty acid content of *Listeria* cells and their dependence on the medium. Todd-Hewitt broth and tryptose broth were used as the basic medium. The lipid content of the virulent India strain corresponded to the values given in the literature for other strains (6.1% of the dry weight). On using Tryptic-Soy-Broth (TSB) it attained 13.7%. The addition of glucose to these media significantly stimulated growth of the bacteria, but did not influence the total lipid concentration. The addition of glycerin significantly raised the lipid content, however — to 10% in the first two media and to actually 18% in TSB medium. The avirulent K₃-Welshimmer strain displayed much lower lipid production (3.7%) than the virulent test strains. The addition of glycerin to the medium raised the lipid content of this strain to 10.6%. Glycerin likewise raised the total fatty acid content of *Listeria* lipids. Paper and gas chromatography showed the presence of 8–10 fatty acids, the reciprocal ratio of which varied in association with the culture medium. A fatty acid with a large number of carbon atoms was detected by paper chromatography.

Differentiation of Pathogenic and Non-pathogenic Escherichia coli Strains Isolated from Piglets and Calves by the Dilatation of Ligated Intestinal Loops Test and the Possibility of Influencing This Reaction by Pre-Contact with a Pathogenic Escherichia coli Strain. E. SALAJKA, L. ULMANN, M. HORNICH, Z. ŠARMANOVÁ, Research Institute of Veterinary Medicine, Brno.

In 1953 De and Chatterje described the dilatation of ligated intestinal loops test in rabbits for differentiating enteropathogenic *Vibrio cholerae* strains from non-pathogenic strains. Three years later, De and Bhattacharja submitted a report on the possibility of using this method for the demonstration of pathogenicity of *Escherichia coli* strains from coliform intestinal infections in children. Rabbits are not suitable for the differentiation of pathogenic and