

Examination of Sensitivity to Antibiotics in Staphylococci Isolated from Subjects Exposed to Chlorotetracycline. E. SCHÖN, V. WAGNER, Z. MANDLÍKOVÁ, V. ZIEGLEROVÁ, M. WÁGNEROVÁ, V. DRŇKOVÁ, Regional Station of Hygiene and Epidemiology, Prague.

The authors examined the occurrence of chlorotetracycline-resistant (CTCR) staphylococci in the skin and nasal mucosa of patients exposed occupationally to the effect of tetracycline antibiotics. They found that a high percentage of these persons harboured CTCR strain (72% of workers in the mixing rooms, 81% of workers looking after chickens, 43% of workers in the slaughter house and 97% of those working in the production of TC antibiotics) whereas in the control group of unexposed subjects the occurrence of CTCR strains was only 4%. They further found that the incidence of CTCR staphylococci increases in dependence on the exposure period, i.e. on the duration of employment. Colonization with the resistant strains occurs soon after starting to work in the exposed section. In subjects exposed for 0–3 months the incidence was 59% CTCR strains, in the group from 3 to 12 months it was 64%, in the groups from 1 to 3 years it was 66%, in the 3–5 year group 80% and over 5 years 84%. The resistant strains even spread to workers who are not directly exposed (clerks, laboratory personnel — 47% CTCR strains). The danger of mass application of antibiotics for feeding domestic animals is discussed from the point of view of increasing incidence of resistant microorganisms and their spreading in the human population and subsequent danger of failure of antibiotic treatment. It is recommended to abolish the use of antibiotics for feeding farm animals or to replace those now used by antibiotics which are not used therapeutically.

Demonstration of Extracellular Toxin of *Listeria monocytogenes*. J. SOUČEK, F. PATOČKA, A. SOUČKOVÁ, Laboratory for Special Medical Microbiology and Immunology, Charles University, Prague.

It has been assumed that *Listeria monocytogenes* produces an extracellular toxic factor (or factors) from the beginning of the more detailed study of its pathogenicity, particularly since the demonstration of histological changes in experimental listeriosis, but no true toxin was isolated nor was a toxic antigenic protein determined. The unconfirmed report of Liu and Baker should be mentioned here. They precipitated the supernatant of very young cultures of *Listeria monocytogenes* with ethanol at low temperature and obtained a product causing a dermonecrotic reaction in rabbit skin. The report of DiCapua *et al.* is also not quite clear. They maintained that chinchillas with implanted diffusible chambers containing virulent cultures of *Listeria monocytogenes* died within

a few days without marked macroscopic changes, the demonstration of listerias in the organs being negative. They assumed that death was caused by the toxin produced by listerias *in vivo*. More recently, attention was turned to listeria haemolysin which was purified and possessed not only striking haemolytic activity but which was also tested for lecithinase activity. The haemolysin appears to possess properties resembling those of C-streptolysin and of toxic phospholipase. In the present experiments we used a type 1 strain of *Listeria monocytogenes* isolated from a human fetus transferred almost 100 times through mouse brain. The LD₅₀ of the strain for mice intracerebrally was repeatedly about 20 bacteria. The haemolytic power of this strain remained quite low. In orientation experiments the centrifuged listerias were disintegrated mechanically and the supernatant after centrifugation injected intradermally into rabbits. A clear erythematous and edematous reaction developed around the injection point within 12 hours. In another series of experiments we used directly the filtrate or the supernatant of Todd-Hewitt broth of the same strains of *Listeria monocytogenes* grown for 12, 24 and 48 hours, at 20 and 37 C. The filtrate was concentrated by precipitation with methanol in the cold and injected intradermally into the rabbit where it caused reddening with oedema, the centre of which underwent haemorrhagic necrosis. The same product was incubated with sphingomyelin from ram erythrocytes and its cleavage into N-acylsphingosyl phosphate and choline was demonstrated chromatographically. According to the cleavage of the substrate and the *in vivo* effect in rabbit skin one can assume that we are dealing here with a toxic enzyme analogous to that previously detected in *Corynebacterium hemolyticum*, *Corynebacterium ovis*, *Corynebacterium ulcerans* and identified as phospholipase D.

Contributing Factors to *Mycoplasma pneumoniae* Produced Stimulation of Rhinovirus-RNA Synthesis. R. D. FLETCHER, W. H. MILLIGAN, J. N. ALBERTSON, Jr., School of Dental Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, U.S.A.

It has been demonstrated that *Rhinovirus ribonucleic acid* (RNA) synthesis was greater in *Mycoplasma pneumoniae* inoculated KB (Human Carcinoma of Nasopharynx) cells than in PPL0-free tissue systems (Milligan, W.H.III & Fletcher, R. D., unpublished data, 1969). This is in agreement with the report of Singer, Kirschstein and Barile (Bacteriol. Proc. V245, 1969) who demonstrated increased vesicular stomatitis virus and Semliki Forest virus yields in *Mycoplasma*-infected hamster cells. In our study, *Mycoplasma pneumoniae* was grown on glass, washed three times in Earle's maintenance medium and resuspended in fresh Earle's medium. This procedure eliminated the PPL0 medium. However, if PPL0 medium was added to the