# III. PROPHYLACTICAL PROBLEMS

## STUDY OF THE PATHOGENETIC CHARACTERISTICS OF LISTERIA MONOCYTOGENES AND LISTERIA INNOCUA STRAINS ON CHICKEN EMBRYO MODELS

#### L. Nicheva, D. Yonkova

The pathogenicity of Listeria strains is determined by their haemolytical ability and biological tests with white mice, guinea pigs and rabbits (5). The degree of sensitiveness of laboratory animals towards any Listeria monocytogenes strain is different (1, 2). The strain virulentness, dose and way of application have their importance. The conjunctival test (Anton-test) is applied to guinea pigs and rabbits and the pathogenic strains provide a kerotoconjunctival reaction in them (3). The injection of 0.2—0.5 ml 24-hour broth culture of Listeria monocytogenes in white mice leads to a quick death in the first days after inoculation. Certain authors consider white mice unsufficiently sensitive towards infections with Listeria monocytogenes and they suggest a cortison treatment some 2—4 hours before inoculation. Basic criterion for application of Listeria innocua is lack of haemolytic activity and apathogenicity for the laboratory animals.

The object of our present work was to study the sensitiveness of various strains Listeria monocytogenes and Listeria innocua (pathogenic and apathogenic for laboratory animals) on a chicken embryo model.

#### Materials and methods

We investigated 3 pathogenic strains Listeria monocytogenes (N. 5006, 5507, 4168) and 7 strains Listeria innocua, apathogenic for white mice, guinea pigs and rabbits (N. 172, 475, 572, 712, 470, 2998, 36). All Listeria strains were isolated from humans (ill and bearers).

The study was held on II-day chicken embryos which were infected amnyotically with a smear of 24-hour agar culture of the correspondent strain. The suspension was standarded to 1 bill/ml, then the dilutions with saline solution were done to  $10^{-1}$ — $10^{-9}$ . The chicken embryos (4 for each dilution) were injected with 0.2 ml of the material. The control group embryos was inoculated with 0.2 ml saline solution. Parallelly was done a glucose-agar culture in the same dose. 24—48 hours after incubation the cultures were set to a counting of their colonies, thus controlling the injected microbial cells in the embryos. The infected chicken embryos were examined until the 12-th day after inoculation; their vitality was every day checked by ovoscopation. Then the dead embryos were opened and materials from their organs were cultured in the enriched medium of Holman. After a 24-hour incubation at  $37^{\circ}$  C they were placed in the medium of Ralovich.

## Results and discussion

The results of our study can be seen on Figure 1. We registered 7 investigations during the 12-day period which made possible to determine the evolution of the pathogenic process in the infected chicken embryos. Certain dif-



Fig. 1

ferences in the dynamics of the infectious process for the various strains were detected. It was interesting to establish, that pathological changes in various degree were registered not only with the 3 strains Listeria monocytogenes but also with the 7 strains Listeria innocua, which were apathogenic for white mice, guinea pigs and rabbits. Thus, for example, strain N. 172 did not provoke any changes in any of its dilutions for the first investigation; all embryos were alive. After the second investigation dead embryos were registered until  $10^{-3}$ , after the fourth investigation — until  $10^{-5}$ , after the fifth until  $10^{-7}$ . After the sixth investigation we registered no dead embryovs. Till the end of all investigations alive embryos were only those treated by last dilutions  $(10^{-8}, 10^{-9})$  and also those infected with strain N. 2998. The latter caused the death of the embryos 1 day after the pathogenic strains and only with higher concentrations of the suspension. The rest studied strains were registered as follows: strain N. 475 caused death of the embryos in concentrations  $10^{-1}$ — $10^{-3}$  after the first investigation. The rest embryos were alive till the end of the examination. Strain N. 572 caused death of the embryos after the third investigation (2 days after the pathogenic strains). Embryos infected with lower concentrations  $(10^{-3})$  were alive till the end of the examination. Strains N. N. 36, 470, 712 caused the death of all embryos due to pathological disorders in thier organs in any concentration and any investigation.

Our data show that chicken embryos are sensitive towards strains Listeria monocytogenes and Listeria innocua. There is a certain pathogeneity even with the apathogenic for laboratory animals strains Listeria innocua. All that makes us suggest the 11-day chicken embryos as a suitable biological model to study the virulentness of Listeria strains.

#### REFERENCES

1. Бакулов, И. А. Листериоз сельскохозяйственных животных. М., 1967. — 2. Зеелигер, Х. Листериоз. М., 1959. — З. Апtоп, W. Kritisch-experimenteller Beitrag zur Biologie des Bacterium monocytogenes. Mit besonderer Berücksichtigung seiner Beziehung zur infektiösen Mononukleose des Menschen.-Zentrabl. Bakteriol., *I. Abt.*, 131, 1934, 89—103. — 4. Kampelmacher, E. A. monocytogenes infections in cortison treated mice. A bacteriologic and histopathologic study.-In: Proc. III. Internat. Symp. Listeriosis. Biltheven, 1966, p. 65.—5. Seeliger, H. P. R., M. Schvofs. Serologic analysis of non-hemolyzing strains of Listeria sp.-In: Proc. VII. Internat. Symp. Listeriosis. Varna, 1977, p. 24.

### ИССЛЕДОВАНИЕ ПАТОГЕННЫХ СВОЙСТВ ШТАММОВ LISTERIA MONOCYTOGENES И LISTERIA INNOCUA НА МОДЕЛЯХ КУРИНЫХ ЭМБРИОНОВ

Л. Ничева, В. Йонкова

#### РЕЗЮМЕ

Исследована патогенность 3 щтаммов Listeria monocytogenes и 7 штаммов Listeria ппосиа, изолированных у больных и носителей этих листерийных штаммов. Исследова ние проведено на моделях куриных эмбрионов, которые являются наиболее подходящими биологическими моделями для изучения вирулентности листерийных штаммов.