

# CHANGES IN THE LUNGS OF ALBINO MICE INFECTED WITH *Escherichia coli*

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At autopsy on patients dying from various diseases, and especially on children with enterocolitis, pneumonia caused by *Escherichia coli* is sometimes found.

The object of this investigation was to study pneumonia caused by experimental infection with *E. coli*.

## EXPERIMENTAL

Experiments were carried out on 531 albino mice which were injected with different strains of *E. coli* (enteropathogenic O<sub>111</sub>B<sub>4</sub>H<sub>2</sub>—No. 84 and No. 6247, and untyped No. 112). Preparations of endotoxin were obtained from these strains by Boiven's method. M. L. D. for strains Nos. 84 and 112 was 0.01 ml, so that they could be classed as highly toxigenic. M. L. D. for strain No. 6247 was 0.3 ml, and this was therefore, a strain of weak toxigenicity.

The animals were infected intranasally under ether anesthesia so that on the average 600 million bacterial cells entered the lungs of each mouse.

At each time of investigation, usually 6 animals were taken in each of the three series of experiments. After aseptic removal, the lungs of four mice were ground and seeded on Endo's medium. The material taken from the remaining mice was investigated histologically. The organs were fixed in 10% formalin solution and then embedded in celloidin-paraffin. To detect the microorganisms sections were stained with azure-eosin and by the Nicolle and Gram-Weigert methods.

## EXPERIMENTAL RESULTS

After infection with the highly toxigenic strain *E. coli* O<sub>111</sub>B<sub>4</sub>H<sub>2</sub>, 32 of the 175 mice died in the first 5 days.

Mass dissemination of the bacilli throughout the lungs, with degenerative changes in the epithelium of the bronchial mucous membrane and penetration of the bacilli into the cytoplasm of individual cells were observed 10 min after infection. Leukocytes were clustered in the interalveolar septa.

After 1-3 h, multiple pneumonic foci containing a leukocytic exudate, with traces of serous fluid and macrophages, appeared in the lungs (Fig. 1, I). Well marked phagocytosis of the microorganisms by leukocytes and, in particular, by macrophages was observed (Fig. 1, II). The number of bacilli found in the foci was considerably increased.

After 6-9 h the size of the foci was increased and they now occupied 30-50% of the total area of the organ. The alveoli and bronchi were filled with a thick exudate consisting mainly of leukocytes. The free-lying microorganisms were much fewer and those which were found were deformed.

After 12 h a second wave of multiplication of the bacilli was observed, and the pathological process showed further progressive development.

The pneumonic foci 24 h after infection occupied about 60% of the total area of the lungs. The character of the exudate remained as before. The number of microorganisms gradually diminished.

On the following days the airless foci occupied 60-70% of the total area of the lungs. Among the cells of the exudate, the macrophages were relatively more numerous. No free-lying microorganisms were seen at this stage of the process.

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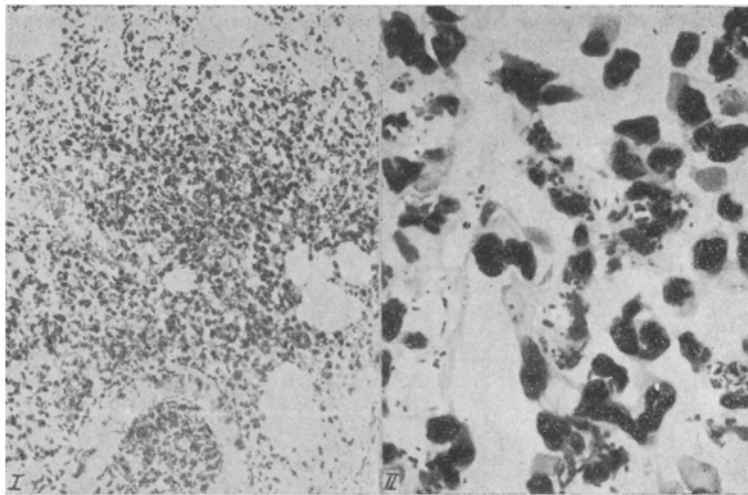


Fig. 1. Pneumonic focus in a mouse 3 h after infection with a strain of *E. coli* with high toxigenic properties. Azure-eosin. I) 270  $\times$ . II) Many microorganisms are visible in an exudate mainly of leukocytes, and are partly phagocytosed. 1600  $\times$ .

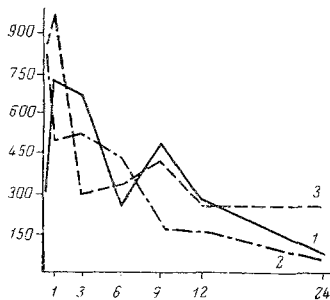


Fig. 2. Dynamics of changes in the number of microorganisms in the lungs of albino mice infested intranasally with strains of *E. coli*. 1) Highly toxigenic strain *O*<sub>111</sub>B<sub>4</sub>H<sub>2</sub>; 2) weakly toxigenic strain *O*<sub>111</sub>B<sub>4</sub>H<sub>2</sub>; 3) highly toxigenic untyped strain. Abscissa) time after infection (in days), ordinate) number of bacterial cells (in millions).

to those in the mice infected with the highly toxigenic strain of enteropathogenic *E. coli*. In half the animals the second wave of multiplication of the microorganisms was actually observed (on the 1st - 2nd day after infection).

The morphological data largely confirmed the results of the microbiological investigation (Fig. 2).

It is clear from Fig. 2 that the dynamics of clearance of the lungs from the pathological agent was dependent to some extent on the toxigenicity of the strains. In particular, 9 h after infection with highly toxigenic strains, the number of microorganisms isolated from the lungs increased, but this did not happen after infection with the weakly toxigenic strains.

Starting on the 4th day, gradual disintegration of the leukocytes and thinning of the exudate took place. From this time no microorganisms were detected. Clearance of the lung tissue then occurred. By the 11th day only residual phenomena after pneumonia were still present in the lungs.

The morphological changes in the lungs of the dying mice demonstrated the severity of the inflammatory process. The fibers in the walls of the large blood vessels became separated and edema developed around them and the pneumonic foci. These changes were caused by the action of the *E. coli* toxin, as was shown conclusively by experiments in which the endotoxin of this microorganism was injected intranasally.

To study the relationship between the morphogenesis of the pneumonias and the toxigenicity of the microorganisms, 99 mice were infected with a weakly toxigenic strain of *E. coli* *O*<sub>111</sub>B<sub>4</sub>H<sub>2</sub>. Four of the animals died in the first 3 days. The character of the morphological changes in these mice was slightly different from that described above, for the second wave of propagation of the microorganisms was absent, the area affected by the lesions was smaller, and fewer mice died at the comparable times.

To examine the possible etiologic role of untyped *E. coli* cells 169 mice were infected with bacilli of this category with highly toxigenic properties; 29 of the animals died in the first 5 days after infection.

The morphological changes in the lungs of these animals were similar

The experiments showed that E. coli, both enteropathogenic and untyped, if injected intranasally into albino mice, causes an infections process in the lungs. The severity of the course of the inflammatory process depends on the degree of toxigenicity of the E. coli but not on its type.