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Pneumonia in White Mice Produced by a Pleuro-Pneumonia-Like Micro-Organism.*

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During the past year, while working with tissues and exudates from patients with rheumatic fever or rheumatoid arthritis, a pleuro-pneumonia-like micro-organism has been encountered in our laboratory mice.

In each instance, normal young white mice of the same breed were inoculated intranasally under ether anesthesia with 0.05 cc of 10-20% tyrode or saline suspensions of human pathologic tissues. However, exudates were introduced without dilution. Serial mouse-passage was carried on at intervals of 4-6 days, using 10-20% lung-suspensions. Blind passages were done in a parallel fashion. Usually by the fourth passage, purple areas of pneumonic consolidation were clearly visible in one or more lobes. In one instance, the pneumonia appeared as early as the second passage. Further passage slowly increased the virulence, morbidity and mortality. Even after months of passage, however, the mortality never increased beyond 20-30%, with death usually occurring on the fourth or fifth day.

Culture of the ground lungs uniformly grew innumerable colonies of a pleuro-pneumonia-like micro-organism. The morphology and

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nature of this microbe has been described in a previous note.[1] Other bacteria were rarely encountered, and these usually disappeared with the next passage. However, in 2 instances, the mortality was highly increased by the association of a gram negative bacillus.

The infective agent was readily preserved by freezing at −80°C. It grew nicely on boiled-blood-ascitic agar plates or broth, in as wide a pH range as 7.0 to 7.8. Multiplication occurred in chick-embryo-tyrode tissue-culture medium,[2] or on embryo-tyrode-agar.[3] We were totally unsuccessful in attaining growth upon the chorio-allantoic membranes of chick embryos in the standard fashion. However, if the inoculated embryo was chilled to death at 4°C, followed by incubation, growth and serial passage were readily accomplished. Organisms grown in this fashion, however, appeared to lose their virulence. Dr. Sabin informs us that two of our strains examined by him are serologically identical with his strain A.[4, 5]

When a large number of mice were simultaneously inoculated with a suspension of the same infective material, it was observed that during the first day there was little change in their clinical appearance except for slight ruffling of the fur. However, even by the end of the first 24 hours, there were small 1-2 mm purple consolidated areas close to the hilar great vessels. Sickness progressed rapidly, with failure to eat or drink, and loss of weight. Some became markedly dyspnoeic. These began to die about the third day, and exhibited almost total pulmonary consolidation. Microscopically, the pneumonia was largely interstitial, with mononuclear phagocytes the dominant cell. Some of these were in mitosis, others actively phagocytic, and a few by fusion formed large giant cells. The consolidated areas were congested. Polymorphonuclear leukocytes were present, especially numerous within the small bronchi. There were a few areas of compensatory alveolar emphysema.

By the seventh day, one of the mice showed a pleuritis. Following this, the survivors became less sick, and began to gain in weight. Periodic autopsy, however, disclosed that some showed small pneumonic areas as late as the twentieth day. On the twenty-sixth day, the entire left lung of one mouse had become pearly gray and cystic in appearance, and the cut surface exuded sticky mucoid material.

1 Dienes, L., and Sullivan, E. R., PROC. SOC. EXP. BIOL. AND MED., in press.

2 Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

3 Zinsser, H., Fitzpatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

4 Sabin, A. B., personal communication.

5 Sabin, A. B., *Science*, 1938, **88**, 189, 575; 1939, **89**, 228.

Similar lesions occur in rats naturally infected with pleuro-pneumonia-like organisms.

When injected intravenously, intraperitoneally or subcutaneously in the two strains of mice at our disposal, our cultures failed to produce any clinical-pathological phenomena. As has been previously noted,[6] however, the susceptibility of different strains of mice is very variable.

Although we encountered this disease and this microbe while working with human rheumatic material, we do not feel justified in concluding that our agent came from aught except mice. Our reasons are these. A similar strain was secured by blind passage of mouse lungs. Using appropriate media, we have been constantly unable to grow it directly from human pathological material, even from uncomplicated fatal active rheumatic autopsy material. Yet such material, when passed through mice, readily yielded the organism.

[6] Dienes, L., and Edsall, G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 740.