

6824

### Studies in Local Immunization of the Lungs of Rabbits with Pneumococcus Type 1.

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Accumulating evidence indicates that an enhanced resistance to bacterial infection may be produced locally as well as generally. To gain further information as to the mechanism of this local immunity we have immunized rabbits intrapulmonically, subcutaneously and intravenously with a pneumococcal vaccine (Type I) and at a later period have tested the resistance of these animals to living pneumococci introduced intratracheally. We have also attempted to determine the fate of the pneumococci, both as to their retention within the lungs and their rates of appearance and disappearance in the blood stream.

Adult male rabbits weighing from approximately 3000 to 4000 gm. were immunized daily for from 4 to 6 days by injections of one cc. of a formalized vaccine of pneumococci. The vaccine was prepared by growing the pneumococci in Kolle flasks and adding 0.2 cc. of formalin to each 100 cc. of the bacterial growth suspended in 0.85% solution of sodium chloride. The intratracheally treated animals were immunized by means of a curved metal tube introduced into the trachea through the mouth; the others were immunized by subcutaneous and intravenous injections of similar amounts of the vaccine. The turbidity of the vaccine was between that in tubes 1 and 2 of the McFarland nephelometer. From 5 to 15 days after the final treatment the animals were infected in groups of 4, consisting of one normal rabbit and an animal immunized by each of the 3 ways mentioned. The 24-hour growth from a blood agar slant of a living pneumococcus culture, Type 1, was suspended in 8 cc. of sterile broth, and 2 cc. was then introduced intratracheally into each of the 4 animals through the metal tube inserted into the trachea. Blood was obtained by cardiac puncture at 5, 10, 15, 30, 45, and 60 minute intervals after infection and cultures made by plating duplicate amounts of one cc. with veal infusion agar, pH 7.6. The presence and numbers of bacterial colonies per cc. of blood indicated the relative degrees of retention of the pneumococci within the lungs and also the rates of appearance and disappearance of the microorganisms in the blood stream. The fatality rates for each group were recorded at the end of one week.

Fifty-one animals were infected intratracheally with the living pneumococci, 17 normal rabbits, 17 immunized intratracheally, 9 subcutaneously and 8 intravenously. Many bacterial colonies appeared in the blood cultures of the normal rabbits as well as in those immunized subcutaneously and intravenously within from 5 to 15 minutes after infection, whereas in most instances, during the entire 60-minute period, none or only a few appeared in the blood cultures of the animals immunized intratracheally. The bacteria disappeared rapidly from the blood stream in the intravenous and subcutaneously immunized animals and much more slowly in the normal rabbits. All of the normal rabbits died within from 12 to 24 hours and at autopsy the lungs were hyperemic and edematous. Three of the subcutaneously immunized and one of the intravenously immunized animals died within 24 hours and their lungs at autopsy resembled those of the normal animals. Only one of the intratracheally immunized rabbits died, and this animal survived for 4 days. The lungs at autopsy were definitely consolidated with their surfaces roughened by fibrin. No appreciable amounts of blood were found in the pericardial cavities of any of the animals. All of the other rabbits survived.

In addition to these experiments, others were performed to show the fatality rates alone. The rabbits were immunized intratracheally and subcutaneously as described above, and their resistance to living pneumococci introduced was compared with normal animals. Seventy animals in all were tested, of which 26 were normal, 26 intratracheally immunized, and 18 subcutaneously immunized. All of the normal rabbits, and 3 of those subcutaneously immunized died, whereas only 2 of the intratracheally immunized animals died.