

between inoculation and death of the controls. At present 58 survivors of the standard dose have been under observation for at least 3 months.

Although many variations in procedure prevent any simple statement of the degree of success obtained, the phenomenon of active immunization of mice naturally susceptible to massive doses of a certain line of leukemic cells appears to be established by the 114 mice that have been immunized to resist the standard dose, in contrast to the 2925 non-immunized mice of the same strain that have been inoculated since 1930 with the same standard dose without a single survivor.

### 7569 C

#### Vaccination of Rabbits Against Intradermal Pneumococcus Infection.

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A vaccine of Type I pneumococcus was prepared by cultivating a highly virulent strain in broth for 24 hours at 37°C. The culture was thoroughly centrifuged, the supernatant broth discarded and the pneumococci suspended in sterile distilled water to give approximately 1000 million per cc. This suspension was heated at 60°C. for one hour, cultured for sterility and preserved with 0.3% tricresol.

Similar vaccines were prepared of Type II and Type III pneumococci.

*Results with Type I Vaccine.* Six adult rabbits were given 1 cc. of Type I pneumococcus vaccine per kilo of weight by subcutaneous injection every 5 days for 5 doses. One week after the last dose all, along with 2 controls, were inoculated intradermally with 0.2 cc. of 18-hour broth culture after the method of Goodner.<sup>1</sup> The controls developed the typical local lesions, associated septicemia, fever, leukocytic changes, etc., and succumbed 4 to 5 days after the inoculation.

All of the 6 vaccinated rabbits survived. Local lesions of slight to moderate severity developed in all with positive cultures of the edema fluid over 1 to 2 days following inoculation which were thereafter sterile. Daily blood cultures were negative in 5 animals;

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<sup>1</sup> Goodner, K., *J. Exp. Med.*, 1928, **48**, 1, 413; 1931, **54**, 847.

in 1 the blood culture was positive 24 hours after inoculation and thereafter negative. Mild to moderate fever with slight leukocytosis developed in all over a period of about 3 days and then reached normal.

Two rabbits were given 2 cc. of the vaccine per kilo every 5 days for 5 doses by subcutaneous injection. When inoculated intradermally one week after the last dose both survived with negative blood cultures throughout. The local lesions were mild with positive cultures of the edema fluid only once 24 hours after inoculation in both animals.

Four rabbits were given 1 cc. of the vaccine per kilo every 5 days for 5 doses by stomach tube and inoculated intradermally 5 days after the last dose. Three developed typical local lesions with the associated septicemia, fever, leukocytic changes, etc., and died about 4 days after inoculation; one developed a local lesion of moderate severity with 2 positive blood cultures but survived.

Four additional animals received 2 cc. per kilo every 5 days for 5 doses by stomach tube. When inoculated intradermally 5 days after the last dose 2 survived after moderate local lesions with 2 positive blood cultures, while the remaining 2 died with severe local lesions, septicemia, etc., about 5 days after inoculation.

Mouse serum protection tests were not conducted because as stated by Goodner and Stillman<sup>2</sup> the 2 factors do not necessarily parallel each other.

*Results with Type II Vaccine.* Two rabbits were given 1 cc. and 2 additional animals 2 cc. of vaccine per kilo every 5 days for 5 doses by subcutaneous injection. Two were given 1 cc. and 2 additional animals 2 cc. per kilo every 5 days for 5 doses by stomach tube. Five days after the last dose all, including 2 controls, were given an intradermal injection of 0.5 cc. of an 18-hour broth culture in 2 injections closely spaced.

Both controls and all vaccinated animals survived. Among the 4 animals receiving subcutaneous injections of the vaccine the local lesions however, were much less severe. Among the 4 receiving the vaccine by stomach tube the local lesions were approximately equal to the controls.

All animals including the controls gave 1 or 2 positive blood cultures during 72 hours following inoculation but thereafter sterile cultures. Among the vaccinated animals and particularly those receiving subcutaneous injections of vaccine, the fever and leukocytosis were appreciably less than observed with the controls.

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<sup>2</sup> Goodner, K., and Stillman, E. G., *J. Exp. Med.*, 1933, **58**, 195.

*Results with Type III Vaccine.* Two rabbits were given 1 cc. and 2 additional animals 2 cc. of vaccine per kilo every 5 days for 5 doses by subcutaneous injection. Two were given 1 cc. and 2 additional animals 2 cc. per kilo every 5 days for 5 doses by stomach tube. All, including 2 controls, were given an intradermal inoculation of 0.5 cc. of an 18-hour broth culture 5 days after the last dose.

All of the vaccinated animals and one control survived while the remaining control succumbed with a severe local lesion, septicemia and associated fever and leukocytic changes. The surviving control developed a moderately severe local lesion with 2 positive blood cultures. The 2 animals receiving subcutaneous injections of 1 cc. of vaccine developed moderately severe local lesions with 2 positive blood cultures but the 2 receiving the 2 cc. dose developed milder local lesions with sterile blood cultures. The 2 animals receiving the 1 cc. dose of vaccine per kilo by stomach tube developed moderate local lesions and positive blood cultures (1 had 2 and the second 4) but ultimately recovered. The 2 receiving the 2 cc. dose per kilo also had moderate local lesions with 2 positive blood cultures but also survived.

Goodner<sup>1</sup> has reported that one intravenous injection of 10 cc. of an extremely heavy suspension of washed heat-killed vaccine of Type I pneumococcus vaccine protected rabbits against the local and associated lesions and found protective antibody in the blood 5 days later. In only 1 rabbit out of 8 was this immunity found to last for 2 months. Stillman and Goodner<sup>2</sup> have also given 6 rabbits intravenous injections of a heat-killed vaccine containing Type I, II and III in equal proportions. All survived when inoculated intradermally after the last injection, whereas 3 controls died 1 to 4 days after inoculation. Additional rabbits given intravenous injections of pneumococcus autolysate developed a lesser degree of resistance to intradermal infection. The active immunity was found highly specific for the homologous pneumococci and very low to heterologous types.

We preferred to use subcutaneous injections because adapted to practical use in the immunization of human beings. Oral immunization was also employed because previous investigations by Ross<sup>3</sup> have shown this route is effective in rats and we have also found it somewhat effective in the immunization of rabbits<sup>4</sup> and monkeys.<sup>5</sup>

<sup>3</sup> Ross, V. J., *J. Exp. Med.*, 1930, **51**, 585; *J. Immunol.*, 1926, **12**, 219; 237; *J. Lab. and Clin. Med.*, 1927, **12**, 566; *PROC. SOC. EXP. BIOL. AND MED.*, 1926, **24**, 273.

<sup>4</sup> Kolmer, J. A., and Rule, A. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **30**, 107; 1933, **31**, 245.

Apparently very large doses of vaccine are required by either route of administration and the resulting immunity is apparently of short duration.

*Summary.* 1. Rabbits have been successfully vaccinated by 5 subcutaneous injections of a heat-killed aqueous suspension of Type I pneumococcus vaccine against fatal intradermal pneumococcus infection. 2. When the vaccine was given by stomach tube about 38% of rabbits survived. 3. With Type II vaccine all animals including the controls survived following intradermal pneumococcus infection but the local lesions, septicemia and associated febrile and leukocytic changes were less marked among the immunized animals and especially those receiving 5 subcutaneous injections of vaccine. 4. All rabbits immunized with Type III vaccine by subcutaneous and oral administration survived along with 1 out of 2 controls following intradermal infection. The local lesions and associated fever and leukocytosis were milder among the vaccinated animals than in the controls and especially among those immunized with subcutaneous injections of vaccine.

### 7570 C

#### Chemotherapy of Intradermal Pneumococcus Infection of Rabbits. Effects of Optochin and Other Quinine Compounds.

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Rabbits were inoculated intradermally with 0.2 cc. of 18-hour broth cultures of a highly virulent Type I pneumococcus after the method of Goodner.<sup>1</sup>

Immediately after 2 rabbits were given 0.005 gm. *optochin base* (Merck) and 2 additional animals 0.01 gm. per kilo by stomach tube and the doses repeated every 6 hours over a period of 3 days. There was no appreciable influence upon the local lesions, temperatures, leukocytic changes, positive blood cultures or cultures of edema fluid. All animals succumbed in from 4 to 5 days.

Two rabbits were given *optochin base* in dose of 0.01 gm. per kilo every 6 hours for 4 doses before intradermal inoculation with pneu-

<sup>5</sup> Kolmer, J. A., and Rule, A. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **31**, 243.

<sup>1</sup> Goodner, K., *J. Exp. Med.*, 1928, **48**, 1, 413; 1931, **54**, 817.