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Infection and Immunity Following the Intracutaneous Inoculation of Scrub Typhus.*

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The question has been raised by some observers as to whether an active immunity in scrub typhus actually occurs. As more knowledge is acquired with the experimental disease, information on this point is accumulating. Workers have observed that when a mouse escapes death following an intraperitoneal inoculation of infectious material, it usually resists infection when challenged subsequently. As no visible evidence of infection may have been observed following the first inoculation it could be argued that the animal was naturally resistant or that it had developed an inapparent infection. This argument would be less valid for the guinea pig which shows evidence of a general infection following an intraperitoneal inoculation by developing fever or by death, or for the rabbit which develops a severe infection following an intraocular inoculation. In the case of the mouse or guinea pig there is an occasional survival following an intraperitoneal inoculation while the rabbit usually survives an intraocular inoculation. Those guinea pigs and rabbits that survive are usually immune and the evidence is accumulating that in the experimental animal at least, an active immunity follows a severe infection.

The experiments which are to be reported bear on this point. The data indicate that a solid immunity in guinea pigs, rabbits, and hamsters can be induced at will following a minimal skin infection.

Rabbits. Six normal rabbits weighing approximately 3 to 4 lb were given 2 intracutaneous inoculations in the abdominal region of 0.1 cc of infected ascitic fluid, diluted 1/20 in broth. The infectious ascitic fluid was obtained from a guinea pig which was sacrificed on the fourth febrile day following the inoculation of the Host 21 mite-strain of scrub typhus. Preparations of the ascitic fluid revealed numerous typical intra- and extra-cellular rickettsiæ.

On the seventh day following inoculation all 6 rabbits showed a small, indurated papule at the site of inoculation. These papules were approximately ½ cm in diameter and slightly raised. They were indurated, with a mild inflammatory reaction about them, and remained so for 2 to 3 days. These lesions did not go on to ulceration, but some showed a small necrotic center and a dry scab. The local lymph glands were not enlarged. These indurated lesions persisted for a period of 6 to 8 days when they gradually regressed and disappeared.

Twenty days following the intracutaneous inoculations, 5 of the rabbits were tested for immunity by the intraocular inoculation of 0.15 cc of infectious mouse blood, diluted 1/30 in saline. Five normal rabbits, similarly inoculated at the same time with the same infectious material, served as controls. The inoculum was obtained from an infected mouse representing the 14th serial passage in mice with the Host 21 mite-strain of scrub typhus. Three mice were also inoculated with this suspension to serve as controls on the infectivity of the inoculum. The three mice died on the 13th day after inoculation showing typical signs of scrub typhus with the characteristic peritoneal exudate containing intra- and extra-cellular rickettsiæ.

All of the 5 control rabbits developed the typical ocular reaction following the intraocular inoculation of infectious material. The average incubation period was 11 days at which time a severe, diffuse conjunctivitis was noted with a circumcorneal injection and a

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considerable degree of turbidity of the aqueous chamber. Pannus appeared about the 12th day in all animals, which progressed to cover the whole cornea from the 28th to the 30th day following inoculation. Turbidity rapidly progressed to a maximum and persisted for more than 30 days. Active signs of infection were present in all animals for a period of approximately 28 days.

The ocular reactions in the animals that had received the intracutaneous inoculation previously were entirely different. All 5 of the rabbits were observed over a period of 30 days and none developed the slightest degree of infection. These animals were solidly immunized following the intracutaneous inoculation.

Guinea Pigs. Seven normal guinea pigs were given 2 intracutaneous inoculations of 0.1 cc each of infectious ascitic fluid obtained from a guinea pig infected with the Host 21 mite-strain of scrub typhus. The inoculations were made in separate areas in the abdominal region. A small indurated nodule developed at either one or both areas of injection and appeared from the seventh to the tenth day after inoculation. These lesions persisted for from 2 to 6 days. Outside of this small skin lesion no other evidence of infection was observed.

Fifteen days after the intracutaneous inoculation the 7 guinea pigs were challenged by the intraperitoneal inoculation of 1.0 cc of infectious ascitic fluid obtained from a guinea pig reacting to infection with the Host 21 mite-strain. Seven normal guinea pigs were similarly inoculated to serve as controls. Temperatures of all of the guinea pigs were taken daily for 24 days. All of the control guinea pigs showed varying degrees of temperature, which is the characteristic picture in guinea pigs following the inoculation of the strain under study. Only one of the guinea pigs that had previously received the intracutaneous inoculation developed a febrile reaction above 103.4°F on the 6th to the 19th and 20th day. None of the other guinea pigs developed a febrile reaction. The difference in the febrile reactions occurring in the guinea pigs that had received the intracutaneous inoculation before challenge and the controls was clear-cut.

Hamsters. Six hamsters weighing from 100-130 g were inoculated intracutaneously with 0.2 cc of mouse ascitic fluid obtained after infection with Host 22 mite-strain. This exudate contained many intra- and extracellular rickettsiæ. Approximately 0.1 cc of exudate was diluted with 2.0 cc of broth and 0.2 cc was inoculated intracutaneously. All 6 hamsters developed a small indurated papular lesion by the 5th day. These lesions persisted for 3 or 4 days and then regressed. The animals showed no other visible appearance of disease. Thirteen days after the intracutaneous inoculation these 6 hamsters as well as 6 controls were inoculated intraperitoneally with the Kostival yolk sac culture (40th passage). Each animal received 1 cc of a 10% yolk sac suspension. All the control hamsters died in from 11 to 17 days with a typical scrub typhus infection, while all the hamsters that had previously received the intracutaneous inoculation survived. It is worth noting that the intracutaneous inoculation was made with Host 22 mite-strain and that the animals were challenged with the Kostival strain. There was a complete cross-immunity between these two strains.

Conclusion. When rabbits, guinea pigs, and hamsters are inoculated intracutaneously with small doses of infectious peritoneal exudate, an indurated papular lesion develops which lasts a few days and then regresses to disappear completely. We have no evidence on the possible distribution of the agent following the intracutaneous inoculation. When these animals are subsequently challenged by the inoculation of infectious material, they are shown to possess a solid immunity, while the controls show signs of active disease or die. These experiments fit in with observations made by others as well as by us, namely, that an active immunity against scrub typhus in the experimental animal can be induced.