# Passage of Shope's Rabbit Fibroma Virus through One-Day-Old Mice.* (23694)

HERBERT T. DALMAT† (Introduced by W. Ray Bryan)
*U. S. Department of Health, Education, and Welfare, Public Health Service, N.I.H., National Institute of Allergy and Infectious Diseases,‡ Bethesda, Md.*

The virus of fibromatosis, like the closely related virus of myxomatosis, is considered specific for rabbits in nature. It produces local lesions in cottontail rabbits (*Sylvilagus* spp.) in the eastern and midwestern United States as well as in domestic rabbits under experimental conditions. To date it has not been successfully propagated in other animals, except on the chorioallantoic membrane of chick embryos(1). Since Andrewes and Harisjades(2) achieved 30 serial passages of myxoma virus in one-day-old mice, an attempt was made to do the same with the fibroma virus. This paper is a report of our preliminary experiments.

*Methods.* One-day-old NIH general-purpose mice were inoculated intracerebrally with 0.01 ml of a 10% suspension (400 infectious doses) of domestic rabbit tumor tissue prepared in serum broth saline and filtered through a Swinney filter after light centrifugation (1,800 rpm for 7 minutes). The fibroma virus used was the Boerlage strain,§ shipped to the author as infectious rabbit testicle which was then passed by serial intracutaneous injections in domestic rabbits. The serum broth saline was essentially that of Andrewes and Harisjades(2). For preparation of mouse brain suspensions, the brains of 2 mice of an infected litter were removed aseptically, weighed, ground in a mortar, suspended in serum broth saline to make a 10% suspension, and then centrifuged lightly. The resultant suspension was then used for preparing dilutions used for inoculating mice intracerebrally or rabbits intracutaneously. Rabbits were usually inoculated in the flanks with diminishing 10-fold dilutions. This served both to indicate positive transfer through the mouse brain and to determine the changes in virus titer incident to such passage.

*Results.* In one experiment, 4, 7, 16, and 31 days after initial infection of a litter of mice, 10% brain suspensions were prepared. Each of several sites on the flanks of a test rabbit were inoculated with 0.25-ml quantities of dilutions of this suspension, calculated to contain either 5 or 50 infectious doses. Tumors resulted at all sites on all the animals except the one inoculated with mouse brain infected for 16 days. In the second similar experiment, tumors developed on rabbits as a result of inoculation with mouse brain suspensions 3, 7, 11, and 26 days after infection but again, not as a result of the inoculation with a suspension prepared 16 days after infection. There was no apparent variation in the time of appearance or rate of growth of the tumors as a result of inoculations at different intervals from the time of mouse infection.

In several experiments involving brain-to-brain passage, transfer was positive in only one series in which 3 serial passages were accomplished. The virus dilution from mouse to mouse was approximately 250-fold, each mouse in the third transfer theoretically receiving only $2.5 \times 10^{-5}$ infectious doses. The titration in a rabbit of the brains of the mice of the third passage was $10^{-4}$ per 0.25 ml, only a 10-fold drop from the titer of the original tumor tissue used. Unfortunately, the mouse brain passage was discontinued during the absence of the author and was unsuccessful when later resumed with some of the frozen brain material of the third passage. In view of the difference in virus yield from the brains of litter mates shown to exist in myxoma passage(2), it is probable that the

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† With the technical assistance of James C. Cunningham.

‡ Laboratory of Tropical Diseases.

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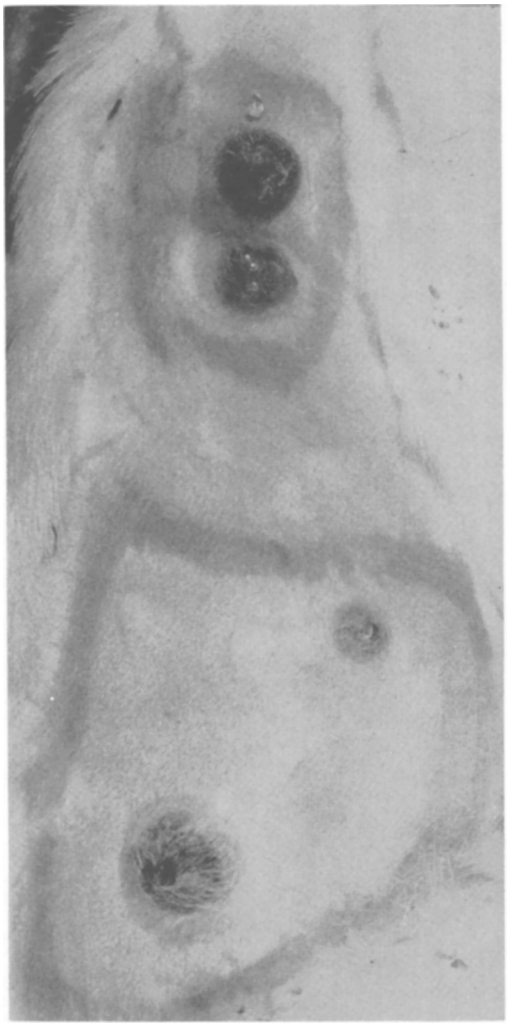

FIG. 1. Fourteen-day fibromas produced by inoculation of suspensions of mouse brains infected 7 days previously. These tumors were infective for mosquitoes when tested at 15 days.

particular tissues that had been preserved were of extremely low titer or completely negative. In all other series, the mouse-to-mouse passages were unsuccessful, although virus was always recovered from the brains of the first litter infected, as shown by inoculations into the skin of a test rabbit. Attempts to establish the virus by intracutaneous and subcutaneous inoculation into mice failed.

Mosquito (*Aedes aegypti*) [L.] transmission was attempted from the 14-day-old domestic rabbit tumors (Fig. 1) resulting from inoculation of a suspension of first passage mouse brain. Normally, mosquitoes are unable to transmit virus from domestic rabbit fibromas while they can from cottontails(3,4). However, in this instance the refeeding of the mosquitoes, as well as inoculation of their mouthparts, resulted in good tumor formation. Transmission was attempted from other tumors resulting from inoculation of first and second passage mouse brain suspension, and these were also successful.

*Summary.* The fact that the virus titer, as shown by intracutaneous inoculations into rabbits, declined only slightly in the 3 serial passages through mice, despite a 250-fold dilution at each passage, indicates the possibility of virus proliferation. However, too few experiments were performed to establish this relationship clearly. There is no doubt that the virus can persist in the brain of the living mouse (one-day-old) for periods at least up to a month. With further attempts at mouse-to-mouse passages it appears likely that the virus can be established in this host.

While fibromas of domestic rabbits that were produced by direct inoculation of tumor suspensions are usually non-infective‖ for arthropods, those tumors resulting from arthropod transmission from the natural cottontail rabbit hosts are often infective(4). Treatment of the recipient domestic rabbits with x-rays or carcinogens prior to infection with the virus also effectively changes the infectivity of the resultant tumors so that they are infective for arthropods(4,5). It now appears that passage of the virus through one-day-old mouse brain, which does not seem to affect the mouse, does alter the virus so that subsequent inoculations into domestic rabbits produce infective tumors.

‖"Infectivity" as used in this paper refers to availability of the virus in the tumors to arthropods as demonstrated by ability of the arthropods to transmit it to a second host.

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