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A Failure to Find Virus III Infection in Rabbit Breeding Stocks.

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Rivers and Tillett[1,2] and Miller, Andrewes and Swift[3,4] had no difficulty in recovering Virus III by serial testicular passage in presumably normal laboratory-stock rabbits. The disease occurred in the 3d, 4th, 5th, or 6th passage in over 30% of trials. Fifteen to 20% of stock rabbits were either refractory to infection or contained neutralizing antibodies in their serum. No other record of the isolation of Virus III has been found. Recently, for use in some contemplated studies on this disease, it seemed desirable to obtain a new strain of the virus from spontaneous cases. The following report deals with that attempt.

Two groups of 8 rabbits of varying age were selected. The initial inoculum consisted of an emulsion of a spontaneous mammary rabbit-tumor. This was injected in 0.4 cc amounts in each testis of the first rabbit of each series. The rabbits were killed at intervals of 4 days, their testes excised under aseptic conditions, ground with sand and sterile salt solution, and injected in 0.5 to 1 cc amounts into the testes of the next of each series. In no instance was there any clinical or histological evidence of Virus III infection. It seemed probable that failure was due to the absence of the virus in the animals used in this laboratory, all of which come from the breeding stock developed by Dr. Wade H. Brown of The Rockefeller Institute. This supposition was confirmed by the fact that of a group of 90 from the same source which were inoculated at various times with Virus III, no rabbit failed to develop either clinical or histological evidence of infection.

Therefore a search was instituted for an infected stock. This was done by testing the sera of sample rabbits for neutralizing antibodies by their ability to inhibit growth of Virus III in tissue-cultures. The occurrence of the typical Virus III inclusion-bodies

1 Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1923, **38**, 673.

2 Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1924, **39**, 777.

3 Miller, C. P., Jr., Andrewes, C. H., and Swift, H. F., *J. Exp. Med.*, 1924, **40**, 773.

4 Miller, C. P., Jr., Andrewes, C. H., and Swift, H. F., *J. Exp. Med.*, 1924, **40**, 789.

in the nuclei of the proliferating cells was used as a criterion for the presence of virus. Andrewes[5] had previously shown that the sera of animals recovered from the experimental infection prevented the appearance of inclusion-bodies and had correlated their presence with infectivity of the culture. We have confirmed this, and have demonstrated that the neutralizing power persists for at least 8 months after clinical recovery. Immune serum diluted 1:32 was found to be capable of preventing the formation of inclusion-bodies. The sera of the tested animals were used undiluted. The cultures were inoculated with the original strain of the virus isolated by Dr. Thomas Rivers and courteously furnished by him. As shown in Table I, 7 groups of rabbits from various sources—a total of 163 individuals—were examined. The groups came from the Brown breeding stock of The Rockefeller Institute at Princeton, New Jersey; from the breeding stock of the laboratories of The Rockefeller Institute in New York City; and from 5 independent rabbitries, 4 in the vicinity of Princeton and one in New York. The animals varied in age from 6 weeks to over 2 yrs. Approximately equal numbers of each sex were used. In the majority of cases they were of mixed breed, but one group of 20 was composed of 14 pure breeds. No wild rabbits were tested. The experiments were performed during the fall and winter months.

TABLE I.

Source	No. of rabbit sera tested in cultures	Approximate no. of rabbits in colony	% tested
Brown stock, Princeton	100	700	14
Rockefeller Institute stock, New York	20	300	7
Independent stock A	11	30	33
" " B	8	50	16
" " C	8	25	33
" " D	8	75	10
" " E	8	20	40
Total	163	1200	14

None of the 163 sera tested showed neutralizing antibodies. If their absence is evidence of freedom from infection, it would appear that Virus III in rabbits is not common. It is of interest that the animals from The Rockefeller Institute laboratories in both Princeton and New York originated in part from the same stock in which Rivers and Tillett, and Miller, Andrewes and Swift, found such a high incidence of infection in 1923.

[5] Andrewes, C. H., *Brit. J. Exp. Path.*, 1929, **10**, 273.