

serum would not be strong enough to serve as a diagnostic test serum. The method is especially recommended for this purpose. The "incomplete" or "blocking antibody" has been found to be associated to a large extent with the supernatant, indicating that at least a partial separation of the Rh agglutinin from the "blocking antibody" has been accomplished by dialysis. The effectiveness of the

resulting Rh agglutination by the globulin fraction is therefore not only attributed to simple concentration of the Rh agglutinins, but also to the partial removal of the Rh "blocking antibody." The isoagglutinins anti-A and anti-B are not concentrated to the same extent as the Rh agglutinins which constitutes an additional advantage of the globulin preparation over the untreated serum.

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Results of Inoculating Okinawan Horses with the Virus of Japanese B Encephalitis.

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It has been reported previously¹ that a considerable percentage of Okinawan horses, from which samples of serum were obtained during an epidemic of Japanese B encephalitis, possessed antibodies against the virus responsible for the epidemic. This observation suggests that horses may occupy the role of intermediary hosts for the virus. The importance of this role in the epidemiology of the disease in human beings depends to a large extent upon whether the virus is capable of circulating in the blood stream of infected horses, from which source it might be transmitted to man by mosquitoes. In order to obtain information concerning the matter, four Okinawan horses were inoculated with the virus of Japanese B encephalitis on 16 September, 1945. The present report is concerned with the results of the experiment.

Materials and Methods. Through the cooperation of the Commander of Naval Construction Troops, a screened, wooden-frame

stable was built on the outskirts of the village of Ishikawa. This building contained four stalls, a separate room for laboratory studies, and a concrete floor with drainage outlets leading to a nearby pit. Throughout the course of the experiment, the interior was thoroughly sprayed each day with DDT.

Four horses were selected from the corral at Ishikawa. Like virtually all other horses on Okinawa, these were malnourished, undersized animals whose general physical condition was obviously poor. Three of the horses were approximately one year old; the fourth was a Mongolian pony approximately 12 years old. Each horse was inoculated intravenously with 2 cc of a 10% suspension of mouse-brain tissue, infected with a strain of Japanese B encephalitis virus isolated during this epidemic from a fatal case of encephalitis.² Specimens of blood were obtained from each horse before the injection of virus and at intervals of 16 hours, 3, 6, 9, and 12 days after inoculation. Neutralization tests were performed with the pre-inoculation sera in order to determine the status of immunity in each animal at the time of injection. The specimens of blood taken after inoculation were injected intracerebrally and intraperitoneally into mice, in order to determine the presence or absence of virus. In the instances where a virus was recovered

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¹ Hodes, H. L., Thomas, L., and Peck, J. L., *Science*, in press.

² Hodes, H. L., Thomas, L., and Peck, J. L., *Proc. Soc. Exp. Biol. and Med.*, 1945, **60**, 220.

TABLE I.
Results of Neutralization Tests and Mouse Inoculations with the Blood of Horses Inoculated with Japanese B Encephalitis Virus.

Horse No.	Neutralizing titer*	Virus in blood				
		16 hr	3 days	6 days	9 days	12 days
1	0	0	+	+	0	—
2	1000	0	0	0	0	0
3	1000	0	0	0	0	0
4	1000	0	0	0	0	0

* Titer is expressed as number of 50% mortality doses of virus against which each serum protected mice.

from the blood stream, it was subsequently identified as that of Japanese B encephalitis by means of neutralization tests in mice.

Results. The results of neutralization tests with sera obtained prior to inoculation, and the results of the injection of mice with blood taken following inoculation, are shown in Table I. It will be seen that Horse No. 1, a yearling, did not possess demonstrable neutralizing antibodies in the serum obtained before inoculation. Sixteen hours after injection, the virus had apparently disappeared from the circulating blood, as shown by the negative results obtained by the injection into mice of blood removed at this time. However, 3 days following inoculation, the virus had reappeared in the blood, since each of 3 mice, which were injected with blood removed on the third day, developed typical symptoms of encephalitis and died 6 days after injection. The virus in the brains of these mice was identified as that of Japanese B encephalitis by means of neutralization tests with known hyperimmune Japanese B antiserum. On the 6th day after the inoculation of Horse No. 1, virus was again detected in the blood of the animal and subsequently identified as the virus of Japanese B encephalitis. On the 9th day, virus was not demonstrated in the blood of Horse No. 1. It is noteworthy that, on this day, its serum was proved to contain sufficient antibody to neutralize 100 50%-mortality doses of virus in mice. It is possible that the failure to demonstrate virus in the blood on this day may have been due to a masking of small amounts of virus by antibody.

Horse No. 1 became ill on the sixth day, failing to eat and showing generalized weakness. The weakness increased during the next four days, and on the 10th day the animal

was moribund. At this time it was sacrificed, and specimens of tissues were obtained for inoculation of mice as well as for histopathological examination. Virus could not be recovered from the brain, spinal cord, or spleen of this horse, and microscopic examination of the brain showed no evidence of encephalitis. The cause of the death of this horse is not known; it is possible that it may have been due in part to prolonged malnutrition.

On the day of the experimental inoculation, Horses No. 2, No. 3, and No. 4, as is shown in Table I, each possessed antibody in sufficient titer to neutralize 1000 50%-mortality doses of virus in mice. As might be expected, virus was not recovered from the blood of any of these animals at any time up to the 12th day after inoculation. These horses developed no symptoms of infection and remained entirely well for a period of 20 days after inoculation.

Summary. It has been demonstrated that an Okinawan horse was susceptible to infection by the virus of Japanese B encephalitis and that the virus was present in the circulating blood 3 and 6 days after inoculation. It could not be demonstrated 16 hours after inoculation, nor after 9 days, at which time a small amount of neutralizing antibody had appeared. In 3 other horses which possessed high titers of neutralizing antibodies prior to the injection, virus could not be demonstrated in the blood stream following inoculation. These results, when considered in conjunction with the previously demonstrated presence of high titers of antibodies in the sera of many Okinawan horses,¹ constitute further evidence that this species of animal may play a role in the epidemiology of Japanese B encephalitis.