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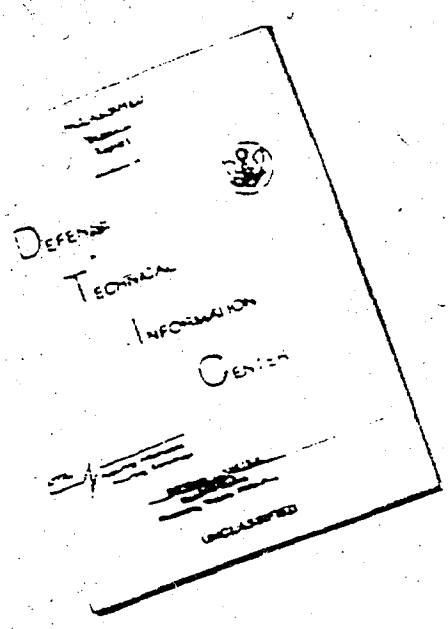

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STUDIES TO DEVELOP AN EXPERIMENT ANIMAL MODEL SYSTEM FOR THE LABORATORY INVESTIGATION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS INFECTIONS

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13. ABSTRACT

Research was conducted to determine the suitability of the hamster (Mesocricetus auratus) as a laboratory animal model for epidemiological and immunological studies on Venezuelan equine encephalomyelitis (VEE) virus infections. The studies were made using the live, attenuated VEE (Strain TC-83) and the live, attenuated western equine encephalomyelitis (WEE) (Clone 15) vaccine viruses. Both viruses elicited measurable hemagglutination-inhibition and neutralizing antibody responses in the hamsters. Little evidence was obtained to suggest that pre-existing antibodies to one virus influenced the response to the other. VEE antibodies were shown to be passively transferred from immune hamsters to their offspring both in utero and through the milk and passively acquired maternal antibodies protected neonatal hamsters against lethal challenge with VEE virus. The maternal behavior of lactating hamsters was highly undesirable, and was characterized by the cannibalism of the young and an absolute refusal to foster-rear foreign young. The incidence of cannibalism was unusually high among females vaccinated with VEE virus; suggesting a mental disturbance. Based on these studies it was concluded that the hamster was unsuitable as a model for investigations on passive transfer of maternal antibodies to VEE virus.

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Vaccine						
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SUMMARY

(Summary is en route to us and will be included in the report when distributed.)

I. INTRODUCTION

An epidemic strain of Venezuelan equine encephalomyelitis (VEE) virus (subtype IB) invaded the United States from across the Mexican border during the summer of 1971 (CDC MMWR, 1971). The infection initially occurred in the Lower Rio Grande Valley in Texas and it spread to several counties of the state over the next several weeks. A variety of control measures, involving state and national resources, were used before the epidemic was finally halted during the early fall of 1971. One of the measures credited with helping to control the epidemic was the use of the live, attenuated, VEE vaccine TC-83.

The TC-83 VEE vaccine was developed by the United States Army during the early 1960's (Berge, et al., 1961). It was originally intended for use in humans, but it was used widely to vaccinate equines against VEE in Central America and Mexico during the period 1969 through 1971 (Spertzel and McKinney, 1972; APhIS Pam. 91-10, 1973). Although still classified as an experimental product, the vaccine proved to be effective in interrupting the transmission of VEE virus in nature and it induced protective immunity in equines against clinical VEE virus infections.

The vaccine was still classified as an experimental product at the time of the 1971 Texas epidemic. The seed virus from which it had been developed was a known equine virulent strain. Thus there was an initial reluctance to vaccinate equines in the United States out of the fear that the vaccine virus might revert to its more virulent form. After the epidemic continued to spread in Texas, and when it appeared that the infection might invade other states, the widespread use of the vaccine was authorized. The vaccine was credited with being a major factor leading to the termination of the epidemic and only one reported incidence of adverse effects was associated with its use.

Following the epidemic the general assumption was made that VEE virus had become established in the United States. After considerable debate and assessment of knowledge it was determined that much remained to be learned of the epidemiology and immunology of the VEE viruses. A limited number of studies had been made on the clinical characteristics of the disease in experimentally infected animals (Victor, et al., 1956; Austin and Scherer, 1971; Gochenour, 1971) and of naturally acquired VEE infections in man (Ehrenkranz, et al., 1970). Other studies had shown that there were endemic and epidemic strains of the virus (Young and Johnson, 1969; Young, 1971) and that these strains behaved quite differently with respect to equine pathogenicity and ecologically (Walton, Brautigam, et al., 1971; Young, 1971). Small mammals (especially certain species of rodents) were thought to be the most likely reservoir hosts of endemic VEE virus strains, but the relationship of these animals to the epidemic strains was unknown. Moreover, a wide variety of mosquito species had been naturally infected with VEE viruses and a number of these had been shown to be capable of transmitting the virus(es) in the laboratory. Yet the precise role(s) of these various species as natural vectors of endemic and/or epidemic strains of VEE virus had not been elucidated.

Finally, a precise biological definition of the various strains of VEE virus(es) had not been made. The designations "endemic" and "epidemic" strains were made for convenience and were based on epidemiological and certain minor

immunological characteristics of strains isolated under given circumstances. Because of this, some controversy existed as to the precise classification of these agents. Some workers (Shope, et al., 1964; Young and Johnson, 1967; Young, 1971) regarded the various VEE viruses isolated to be distinct agents, and Shope and coworkers (1964) proposed that the related viruses be grouped into a "VEE Complex". Other investigators (Scherer and Pancake, 1970) maintained that the various agents were not sufficiently distinct to be considered separate viruses and suggested that they be regarded as strains of a single (VEE) virus. Chamberlain (1971) offered the suggestion that the various entities might indeed be distinct at this time, but that they might have all originated from a common virus and that the present differences might have resulted from geographical isolation of strains and/or selective pressures imposed by different vector and host species. Thus the debate goes.

The foregoing discussion points to the need for a suitable laboratory animal model system for use in the study of VEE virus infections. VEE has suddenly been realized to be, perhaps, the most important arboviral zoonotic disease in the Americas. The epidemiology of epidemic strains of the virus has proved to be complex and much remains unknown concerning the origin of these agents, their ecology, their mode of spread, and their immunological characteristics. The availability of a suitable laboratory animal host would provide a means for the detailed study of some of these factors.

A desirable experimental animal model species should simulate the natural (epidemic) host in certain biological characteristics. A host for VEE studies should, therefore, resemble equines with respect to susceptibility to infection; clinical and pathological manifestations of infection; and immunological responsiveness. The model species should also be inexpensive to purchase and maintain, and should lend itself readily to a variety of laboratory manipulations.

None of the commonly used small laboratory animals that have been adequately studied is sufficiently similar to equines to fulfill the above criteria (Victor, et al., 1956; Taber, et al., 1965; Gochenour, 1971; Iida, et al., 1973; Kiuchi, et al., 1973). The remaining common laboratory animal species left for consideration was the Syrian (Golden) hamster (Mesocricetus auratus). This animal has been used widely in biological research. It is inexpensive to purchase, hardy, easy to maintain and manipulate under laboratory conditions, and is very susceptible to lethal infection with VEE viruses (Austin and Scherer, 1971). The pathogenesis of attenuated VEE virus (TC-83) in the hamster is such that the animal survives long enough to mount an immune response (Austin and Scherer, 1971) and the clinical and pathological manifestations of VEE in this species are similar enough to those in equines to allow for meaningful comparisons. However, the precise nature of the hamster's immune mechanism was unknown. This aspect of the biology of the hamster had not been reported and, although it is a rodent, biologists had cautioned against the extrapolation of characteristics from other rodents to this species (Hoffman, 1968). The hamster had been found to be biologically unique in many respects and it was deemed unwise to discount a possible difference in its immunological mechanism.

As a result of the above reasoning the hamster was selected as a candidate experimental animal model to study VEE virus infections. The present studies were designed to gain knowledge of the humoral immune response to the TC-83 strain of VEE virus. The specific objectives of the study were:

1. To determine the capacity of TC-83 vaccine to elicit a humoral antibody response in hamsters.

2. To determine the mode of transfer of maternal antibodies from immune hamsters to their offspring.

3. To determine if maternally derived passive antibodies were protective in newborn hamsters against lethal infection with TC-83 VEE virus.

4. To determine the influence, if any, of antibodies for heterologous Group A arboviruses on the production of a humoral antibody response to TC-83 vaccine virus.

5. To determine the influence, if any, of antibodies for VEE virus on the production of a humoral antibody response to a heterologous Group A arbovirus vaccine.

II. MATERIALS AND METHODS

A. Viruses

VEE virus used in these studies was the TC-83 strain vaccine virus (Berge, et al., 1961). Western equine encephalomyelitis (WEE) virus consisted of Clone-15 WEE vaccine virus (Johnson, 1963). The vaccines were reconstituted in the diluents provided by the manufacturers and then diluted 1:2 in borate buffered saline (BBS) at pH 9.0 containing 0.75 percent bovine albumin (BA) Fraction V, and held frozen at -70 C until used.

VEE and WEE viruses were assayed as described previously by plaquing on monolayer cultures of Vero and duck embryonic cell cultures (DECC), respectively. The titer of the TC-83 VEE virus was $10^{5.7}$ plaque forming units (PFU) per ml and the titer of the Clone-15 WEE virus was $10^{8.72}$ PFU per ml.

VEE virus was also titrated by intraperitoneal (ip) inoculation of suckling hamsters (SH). Serial 10-fold dilutions of virus from the 10^{-1} to 10^{-10} were made in BBS-BA (pH 9.0) and each dilution was inoculated ip (0.03 ml per hamster) into a litter of SH. Animals were observed for signs of illness and/or death over a 14-day period. Virus titers were calculated according to the method of Reed and Muench (1938) and expressed as $SHipLD_{50}$. The $SHipLD_{50}$ of the TC-83 vaccine was $10^{5.5}$ per ml.

B. Serologic tests

Adult hamsters were bled by cardiac puncture and prenatal and newborn hamsters were bled by severing the heart and/or axillary vessels. Blood from adult hamsters was diluted 1:5 by drawing 0.2 ml of blood into syringes containing 0.8 ml of 8.85 percent saline. After clotting the blood samples were centrifuged and the supernates were collected and regarded as 1:10 serum dilutions. Blood samples from prenatal and newborn hamsters were collected by aspiration into nonheparinized capillary tube pipettes. The blood was transferred from the pipettes to 12 x 75 mm plastic tubes, centrifuged, and sera were drawn off and stored as whole serum. All serum samples were held at -20 C until used.

HI tests were conducted according to the method of Casals (1967) and SDN tests were conducted according to the method of Earley and coworkers (1967). The methods used in the conduct of virus challenge studies are described under the appropriate heading of Section C below.

C. Design of studies

1. Serological response of hamsters to Group A arbovirus vaccines: This study was designed to determine the capacity of Group A arbovirus vaccines to elicit humoral antibody responses in adult hamsters. Twenty-one adult hamsters were inoculated ip with $10^{4.7}$ PFU of VEE virus and 12 hamsters were given $10^{7.72}$ PFU of WEE virus ip. In each instance the animals were inoculated with 1/10 the recommended equine dose of vaccine. Because of the high mortality experience among the initial hamsters inoculated with the VEE vaccine subsequent groups of hamsters were inoculated with only $10^{2.5}$ PFU of TC-83 virus. This represented 10^3 $SHipLD_{50}$ of this virus. No undesirable effects were encountered with the WEE virus vaccine and the same dose ($10^{7.72}$ PFU) was used in all studies. Hamsters were bled 1 month (30 days) after vaccination and the sera were tested for the presence of HI antibodies. An insufficient amount of serum was collected to perform both HI and SDN tests as the hamsters were to be used in subsequent breeding experiments. The objective was to ascertain if the vaccines would induce humoral immune responses. In subsequent experiments sera were examined for both HI and SDN antibodies to show that hamsters responded to Group A arbovirus vaccines.

2. Mode of transfer of maternal antibodies: The purpose of these studies was to ascertain the mode of transfer of maternal antibodies (in utero and/or through the colostrum and milk) from immune hamsters to their offspring. The protocol called for the simultaneous breeding of VEE immune and nonimmune hamsters. Newborns to immune mothers were to be taken from their dams at the time of birth and prior to nursing and foster-reared by nonimmune dams. Young of nonimmune hamsters were, in turn, to be foster-reared by the immune hamsters. When of sufficient size (1 to 2 weeks after birth) the young were to have been bled and their sera assayed for VEE antibodies. The presence of antibodies in the sera of young hamsters reared by nonimmune dams would indicate that antibody transfer occurred in utero. Antibodies in sera of young hamsters foster-reared by the immune mothers would indicate that antibody transfer had taken place through the colostrum and/or milk.

The original protocol could not be followed due to the absolute refusal of lactating hamsters to foster-rear foreign young. A variety of techniques were employed in an attempt to encourage foster-rearing, but none proved successful. Among the techniques employed were the spraying of alcohol on the nostrils of mother hamsters to alter the sense of smell; the sponging of newborn hamsters with alcohol in an attempt to remove (or mask) foreign odors; and, the separation of mothers and young, changing the bedding and exchanging equal numbers of young from different mothers before returning the mothers to the cages. Regardless of the technique employed, mother hamsters systematically detected the foreign babies interspersed among their own and removed them by cannibalism.

The inability to induce hamsters to foster-rear foreign young necessitated a change in the research protocol. Near-term females were delivered by caesarian section and blood samples were taken from the fetuses by cardiac

incision and/or by severing the axillary vessels and aspirating pooling blood into capillary tube pipettes. The samples obtained by either method were less than desirable. The small hearts of the fetuses were ineffective in supplying adequate quantities of blood for use in serological tests and the samples obtained from the axillae were very highly diluted with tissue fluids. In one instance the young of 1 litter were decapitated and efforts were made to obtain enough blood for testing by collecting the drippings. This method also proved inadequate. Sera obtained from these samples were stored at -20° C until tested.

3. Determination of the protective capacity of maternally transferred antibodies against infection with lethal doses of TC-83 VEE virus:

Newborn (2 to 4 day-old) hamsters born to VEE immune hamsters were inoculated intraperitoneally with 1,000 SH LD_{50} of TC-83 VEE vaccine virus. Newborns of nonimmune hamsters were inoculated with the same dose of VEE virus and were used as controls. The immune and nonimmune dams were bled at the time of challenge and the sera were assayed to determine the VEE antibody status of these animals at the time of challenge of the young. Challenged hamsters were observed over a period of 14 days for signs of illness and/or death. Deaths that occurred within 24 hours after inoculation were attributed to technical causes and were discounted. It had been shown through preliminary studies that nonimmune newborn hamsters died consistently within 48 hours after being inoculated with $10^{2.0}$ PFU of the TC-83 VEE vaccine virus. Based on these observations, the survival of young hamsters born to VEE immune dams for at least 2 days longer than the controls following challenge was taken to indicate the presence of protective, passively acquired, VEE antibodies.

4. Influence of heterologous antibodies on the production of a humoral response to VEE or WEE virus vaccines:

Three groups of hamsters were used in this experiment. One group that had been previously immunized against VEE was inoculated with Cone-15 WEE vaccine. A second group consisted of hamsters previously immunized against WEE and then inoculated with TC-83 vaccine. The third group consisted of animals that had no history of previous immunization. These animals were inoculated simultaneously with TC-83 VEE and Clone-15 WEE vaccines. Controls for the WEE vaccines were hamsters that had been inoculated with Cone-15 vaccine in the experiments of Section C (1) at a time when they had no history of previous immunization. Likewise, controls for the hamsters that received VEE vaccine in the present experiment consisted of animals that had been inoculated with TC-83 in the experiments of Section C (1). Comparisons were made between the humoral immune responses manifested by the hamsters with and without preexisting antibodies for group-related arboviruses after vaccination with VEE or WEE vaccines. VEE and WEE conversion rates and heights of antibody responses of the hamsters used in the experiments of Section C (1) were also used as controls for those exhibited by the nonimmune hamsters in the present experiment following the simultaneous inoculations with TC-83 VEE and Clone-15 WEE vaccines. All hamsters were bled at 14 and 28 days post-vaccination and the sera were tested for VEE and WEE antibodies. The percent of animals that developed antibodies and the geometric mean (Gm) antibody titers attained were compared with those of animals that had no heterologous antibodies at the time of immunization.

III. RESULTS

A. Serological response to Group A arbovirus vaccines

Five (24 percent) of 21 hamsters inoculated with $10^{4.7}$ PFU of TC-83 VEE vaccine succumbed within 5 to 10 days after vaccination. The symptoms and signs exhibited by the animals prior to death were suggestive of those previously reported for VEE in hamsters resulting from infection with TC-83 virus (Austin and Scherer, 1971) and the deaths were attributed to fatal VEE infections. Virus isolation and/or pathological studies were not made to confirm the diagnosis.

Fourteen of the 16 surviving hamsters (88 percent) developed VEE HI antibodies within 1 month after vaccination. Positive titers ranged from 1:20 to 1:320 with a Gm titer of 1:59 and a median (Md) titer of 1:40. None of the animals developed cross-reacting WEE or EEE HI antibodies. The results are summarized in Table 1.

Because of the high mortality rate encountered with the above group of hamsters a second group of 15 animals was vaccinated ip with $10^{2.5}$ PFU of TC-83 VEE virus. The mortality rate was 10 percent. The HI and SDN antibody results from this experiment are presented in Table 2. Sixty-four percent of the 14 surviving animals converted to positive for VEE HI antibodies and 71 percent converted to positive for VEE SDN antibodies. HI antibody titers ranged from 1:10 to 1:320 with a Gm titer of 1:40 and a Md titer of 1:20. SDN titers ranged from 1:10 to 1:1,280 with a Gm titer of 1:218 and a Md titer of 1:80.

The HI antibody responses in hamsters vaccinated with Clone-15 WEE vaccine are summarized in Table 3. Eleven hamsters had developed WEE HI antibodies by 1 month after vaccination. Titers ranged from 1:80 to $\geq$ 1:640. The Gm titer was $\geq$ 1:363 and the Md titer was 1:320. None of the animals developed cross-reacting VEE or EEE HI antibodies.

B. Mode of transfer of maternal antibodies to newborn hamsters

Pooled blood samples from 20 litters of prenatal hamsters were tested for VEE HI and SDN antibodies. The sample from only 1 litter gave evidence of possessing VEE HI antibodies and samples from three other litters were positive at low titer for VEE SDN antibodies. The 1 sample possessing HI antibodies gave a $\pm$ reaction in the 1:20 dilution. The mother of this litter had a VEE HI titer of 1:40. In the SDN test 2 litters had titers of 1:10 and 1 had a titer of 1:20 and their mothers had titers of 1:40 and 1:80, respectively.

These results can only be regarded from the qualitative point of view and cannot be considered representative of the true antibody status of the prenatal hamsters. The samples were known to have been highly diluted with tissue fluids at the time of collection. Consequently, these findings provide little more than positive evidence that hamsters, like other laboratory rodents, transfer at least some antibodies passively to the young *in utero*.

C. Determination of the protective capacity of passively acquired maternal antibodies in newborn hamsters

In this study 2 to 4 day-old nursing hamsters were challenged with $10^{2.5}$ PFU of TC-83 VEE vaccine virus. The results of the challenges are presented in Table 4. Of 37 young born to VEE immune mothers 30 (87 percent) survived for at least 6 days after challenge and 23 (77 percent) survived for more than 14 days after challenge. All but 1 of 10 newborns of unvaccinated control hamsters succumbed to VEE within 48 hours after challenge and this animal had succumbed by 72 hours post-challenge. HI antibody titers of the mothers ranged from 1:20 to 1:320 and SDN titers ranged from 1:1,280 to greater than 1:10,240. Five young born to Hamster No. 661 and 2 born to Hamster No. 603 succumbed by the second day after challenge. Both of the mothers had high levels of VEE HI and SDN antibodies at the time of challenge. The time of death of these young was the same as that for the controls. No explanation for the failure of the young to survive the challenge is immediately apparent. It must be assumed, however, that they did not receive antibodies from their dams as the other newborns from immune mothers survived. The results show unequivocally that passively acquired maternal VEE antibodies protect neonatal hamsters against fatal infection with lethal doses of TC-83 VEE virus.

Serum samples were collected from the surviving neonatal hamsters 21 days after challenge to determine if the challenge dose of virus had served to immunize the young animals. Sera from the animals in each litter were pooled for testing. In those instances where litters consisted of more than 4 hamsters pools were made from the sera of 3 animals each. From Table 4 it can be seen that none of the litters possessed VEE HI antibodies and only 2 possessed VEE SDN antibodies. The SDN titer in the young of Hamster No. 638 was 1:2,560 and that in the young of Hamster No. 663 was 1:20. The SDN titer in the young of Hamster No. 638 was only 2-fold lower than that of the mother. Because of the pooling of sera it could not be determined if 1 or more of the animals had responded to the challenge virus, or if the young comprising this litter had received an unusual quantity of passive antibodies from their dam. The results suggest that, in general, passively acquired maternal antibodies prevented the active immunization of the neonatal hamsters by the TC-83 virus, or, that neonatal hamsters lack the capacity to mount a significant humoral antibody response to the TC-83 vaccine virus.

D. Influence of antibodies for a group-related heterologous virus WEE on the production of a humoral response to TC-83 VEE vaccine virus

It was desirable to know if the presence of antibodies to another Group A arbovirus would influence (interference or enhancement) the elicitation of a humoral antibody response to the TC-83 vaccine virus. To investigate this possibility 5 adult hamsters that had been vaccinated 7 months previously with a single inoculation ($10^{7.72}$ PFU) of Clone-15 WEE vaccine were vaccinated with $10^{2.5}$ PFU of TC-83 VEE vaccine. The animals were bled at 14 and 28 days after VEE vaccination and their sera were tested for HI and SDN antibodies against VEE and WEE viruses. The results of these tests are presented in Table 5. All animals possessed WEE HI antibodies at the time of VEE vaccination and 3 had low WEE SDN antibody titers. One hamster (No. 832) also possessed VEE HI and SDN antibodies with titers of 1:20 and 1:80, respectively. This animal had no history of VEE vaccination and had inadvertently become immunized to VEE through aerosol or contact infection.

At 14 days post-vaccination 3 of the 4 hamsters that were initially negative for VEE antibodies had developed VEE HI and SDN antibodies. HI titers ranged from 1:40 to 1:160 and the SDN titers were all 1:320. The HI titer in Hamster No. 832 had increased 2-fold to 1:40, but the SDN titer remained at 1:80. By 28 days post-vaccination the VEE titers had decreased 2-fold in 3 animals and remained the same in the fourth animal. The SDN titers in 2 animals (Nos. 831 and 832) remained at the 14-day level but had decreased by 2-fold to 1:160 in Hamster No. 840 and by 4-fold to 1:80 in Hamster No. 842. Hamster No. 839 did not respond to the VEE vaccine. VEE HI antibody titers increased in all 5 WEE immune hamsters 14 days after VEE vaccination whereas titers of WEE SDN antibodies decreased in 2 of 3 animals.

By 28 days post-vaccination the WEE HI antibody titers in all hamsters were at 1:320. This represented a 2-fold increase in titer for 1 animal and 2-fold decrease in titer for 3 animals. The SDN titer in Hamster No. 840 had increased from $<$ 1:10 to 1:10, but that in No. 842 had dropped from 1:10.

The results of this study showed that hamsters possessing heterologous (WEE) antibodies still retained the capacity to produce antibodies in response to TC-83 vaccine. A comparison was made between the 28-day Gn HI titer in these animals and that attained in the group of hamsters that were negative for heterologous antibodies at the time of VEE vaccination and no statistically significant difference was found. Moreover, the range in titers was similar in both groups. Thus it can be concluded that the presence of WEE antibodies was without effect on the VEE HI antibody response in these hamsters. VEE SDN antibody titers were not determined for the former group. It must be noted that the WEE SDN antibody titers were very low in the animals in this experiment and this prevented a valid evaluation of the influence of these antibodies on the VEE SDN response. It was of interest to observe that the WEE HI titers in these hamsters were boosted by the VEE vaccine while WEE SDN titers were not significantly affected.

E. Influence of VEE antibodies on the production of a humoral antibody response by a group-related heterologous (WEE) virus vaccine

Next it was desired to learn if antibodies against VEE virus interfered with, or enhanced, the production of humoral antibodies against WEE virus. To investigate this possibility 8 VEE immune hamsters were cross-immunized with Clone-15 WEE vaccine. The same hamsters had been immunized 7 months previously with $10^{4.7}$ PFU of TC-83 VEE virus inoculated intraperitoneally. All hamsters were bled at 0, 14, and 28 days after vaccination with WEE virus.

The results of serological tests for VEE and WEE antibodies are presented in Table 6. All but 1 animal was positive for VEE HI and SDN antibodies at the time of vaccination with WEE virus. HI titers ranged from 1:20 to 1:160 and SDN titers ranged from 1:40 to 1:2,560. None of the animals possessed WEE antibodies at the time of vaccination. At 14 days post-vaccination 7 hamsters had produced WEE HI antibodies in response to the Clone-15 vaccine but only 3 had produced WEE SDN antibodies. One animal (No. 813) had a WEE HI titer of 1:80 and the remaining 6 had titers of 1:160. A 14-day sample was not available for Hamster No. 817. Three animals were positive for WEE SDN antibodies at a titer of 1:10.

By 28 days post-vaccination HI antibodies had increased 2-fold in Hamster No. 813 and had decreased 2-fold in Hamster No 815. HI antibodies decreased 16-fold in Hamster No. 820. This was a dramatic drop and one for which no ready

explanation would be made. Hamster No. 817 which had no 14-day sample had a WEE HI titer of 1:320 in the 28-day sample. Only 2 hamsters (Nos. 817 and 821) possessed WEE SDN antibodies at 28 days post-vaccination. Hamsters Nos. 815, 816, and 825 had reverted to negative with WEE SDN titers $<$1:10.

Three hamsters had increases in VEE HI antibodies and 4 had increases in VEE SDN antibodies 14 days after WEE vaccination. SDN antibodies decreased 4-fold in 1 animal (No. 813) and 2 showed no change in VEE antibody levels. By 28 days after WEE vaccination VEE HI antibodies had increased 2-fold above the 14-day level in Hamster No. 820 and VEE SDN antibodies had increased 4-fold above the 14-day level in Hamster No. 828. Hamster No. 817 had a VEE HI titer of 1:320. VEE SDN antibodies were 2-fold lower in Hamsters Nos. 815 and 820 at 28 days post-vaccination than at the time of WEE vaccination. Hamster No. 816 remained negative for VEE antibodies throughout the course of this experiment.

No statistically significant difference was found between the Gm WEE HI titer of the hamsters in this experiment and that for the comparison group (Table 2). The animals in the comparison group were negative for VEE antibodies at the time of WEE vaccination. All animals in each experimental group developed WEE HI antibodies following vaccination with Clone-15 WEE vaccine and this suggested that the presence of VEE antibodies has no effect on the WEE HI antibody conversion rate following the vaccination of hamsters with Clone-15 WEE vaccine. SDN test results could not be compared as the samples from the comparison group were not tested for WEE SDN antibodies.

Further studies on the possible influence of Group A arbovirus antibodies on VEE and WEE humoral antibody responses were made by inoculating hamsters simultaneously with both the TC-83 VEE and Clone 15 WEE vaccines. Eight hamsters having no history of previous immunization with either vaccine were inoculated simultaneously (ip) with the established doses of the 2 vaccines. The animals were bled at 0, 14, and 28 days after vaccination and the sera were tested for HI and SDN antibodies against VEE and WEE viruses.

None of the hamsters possessed antibodies to either VEE or WEE virus at the time of vaccination (Table 7). At 14 days post-vaccination 5 animals had developed VEE HI and SDN antibodies. HI titers ranged from 1:20 to 1:160 (Gm 1:61) and SDN titers ranged from 1:320 to 1:640 with a Gm titer of 1:368. By 28 days post-vaccination sera from the same 5 hamsters were postive for VEE HI antibodies. The titer in 1 (no. TPM 2) had increased by 2-fold, but that in 2 animals (Nos. TP 1 and 847) had decreased by 2- and 8-fold, respectively. The VEE Gm HI titer had decreased from 1:61 to 1:40. Two hamsters had increases in VEE SDN antibodies and 3 had decreases.

Eight (100 percent) of the hamsters developed WEE HI antibodies and 5 (63 percent) developed WEE SDN antibodies by 14 days after vaccination. WEE HI titers ranged from 1:40 to $\geq$1:640 with a Gm titer of $\geq$1:174. WEE SDN titers were at 1:10 and 1:20 with a Gm titer of 1:11. Seven (88 percent) of the 8 hamsters had increases in WEE HI antibodies in the 28-day sample over the 14-day sample levels. Increases ranged from 2-fold to 8-fold and the WEE Gm titer increased from $\geq$1:174 to $\geq$1:452. Three animals (Nos. TP 1, TP 4, and 847) had 2-fold increases in WEE SDN antibodies and 3 (Nos. TPM 1, 845, and 849) had 2-fold decreases in WEE SDN antibodies. There was no change in the WEE Gm SDN titer from 1:11.

In summary, this study showed that hamsters responded to 2 Group A arbovirus vaccines administered simultaneously. However, the type of antibodies (HI or SDN) elicited in largest quantities differed for the two vaccines. The TC-83 VEE vaccine stimulated the production of higher levels of SDN than HI antibodies whereas the Clone-15 WEE vaccine stimulated the production of higher HI than SDN levels. Three (38 percent) of the 8 hamsters failed to produce either HI or SDN antibodies after vaccination with the VEE vaccine, but all (100 percent) animals produced WEE HI antibodies and 7 of the 8 (88 percent) produced WEE SDN antibodies within 28 days after vaccination with Clone-15 WEE vaccine. The results suggest that pre-existing heterologous antibodies for Group A arboviruses might interfere with the production of a humoral antibody response after vaccination with Group A arbovirus vaccines. They further suggest that the antigenic determinants for HI and SDN antibodies are different (or are in defferent locations on the virion), thus allowing one type of antibody to be suppressed without affecting the other.

IV. DISCUSSION

The response elicited by hamsters subsequent to infection with the TC-83 strain of VEE virus has resembled, in many ways, the response of equines to infection with epidemic VEE virus strains. The reported mortality rates have varied from 10 percent to 20 percent (Scherer, 1971; Austin and Scherer, 1971). Most infected animals survived and developed a humoral antibody response (Austin and Scherer, 1971; Zarate, 1971). The clinical VEE syndrome in animals that became ill was characteristic of a viscerogenic, rather than an encephalitogenic, malady. Austin and Scherer (1971) reported the pathology of VEE infection in hamsters was limited largely to the lymphoreticular organs and tissues. Of a large group of hamsters with VEE studied by these investigators, observed signs were rarely referable to the central nervous system. Even in these instances, however, the signs were more suggestive of degenerative lesions (hind limb paralysis) rather than encephalitis. In only 1 animal were histopathological changes found suggestive of encephalitis, and these were equivocal. In summary, VEE infection in hamsters, as in other animals that have been studied (Victor, et al., 1956; Gochenour, 1971) and in man (Johnson, et al., 1968) appears to be primarily an infection of the lymphoid tissues.

The hamster was favored as an experimental model species for other reasons than having clinical and pathological manifestations of VEE virus infections similar to equines. Its hardiness and utility as a sentinel animal for VEE virus activity in nature had been demonstrated by Scherer and his coworkers (1971). It is an inexpensive species to purchase and maintain under laboratory conditions. It requires a minimum of space and it is not subject to a wide variety of spontaneous diseases. The gestation period for hamsters is the shortest (16 days) among established laboratory animal species and they can be bred to produce litters at 2 month intervals. The average litter size is 6 young and these can be weaned at 3 to 4 weeks of age. Hamsters attain sexual maturity at 6 to 8 weeks of age and have an average life span of 2.5 years (UFAW Handbook on the Care and Management of Laboratory Animals, 1967). All of the above characteristics are desirable in a laboratory animal species that is to serve as a model for the study of an acute infectious disease.

Certain critical aspects of the biology of the hamster had to be determined before it could be considered the "ideal" model species for the study of the VEE vaccine virus. These were largely concerned with the maternal behavior of lactating females and the mode of transfer of antibodies from immune mothers to their offspring. Apparently, these biological characteristics of the hamster had not been studied, despite the recent publication of a rather voluminous and otherwise comprehensive work on the biology of the hamster and its utility in medical research (Hoffman, 1968). A knowledge of these characteristics was deemed important as the intended use of the model involved studies designed to elucidate the immunology and epidemiology of VEE virus infections in nature.

Initially, studies were made to confirm that hamsters responded to infection with Group A arboviruses with the production of humoral antibodies. Some workers (Cole and McKinney, 1971) had reported that these animals were poor responders. These investigators studied the serological response in hamsters following vaccinations with VEE, WEE, and EEE virus vaccines. They reported that the HI, SDN, and CF antibody levels that developed were too low and inconsistent for useful analyses. In the present studies no difficulties were encountered in inducing humoral antibodies against either the TC-83 VEE or the Clone-15 WEE vaccines. Both were attenuated virus vaccines.

With respect to maternal behavior, the hamster proved to be a very disappointing species. These animals are very nervous during the few days before and after parturition and are prone to cannibalize their young with only the slightest provocation. It was found that such provocation may include noise, handling the young, disturbance of the mothers, or foreign odors on the young. After considerable experience it was found necessary to isolate expectant hamsters in quiet, dimly-lighted, rooms for periods ranging from 2 to 3 days prior to delivery to 3 to 4 days after delivery in order to minimize the risk of cannibalism. Even with these precautions cannibalism was not eliminated completely.

Another, and perhaps the most serious, characteristic of hamsters--at least from the standpoint of these studies--was the discovery that these animals are not suitable for use as foster mothers. Hamsters absolutely refused to rear young other than their own and foreign young were excluded from litters through cannibalism. Thus it was not possible to return rejected newborns to their natural mothers for rearing.

After unsuccessfully exploiting a variety of techniques, and after losing a significant number of newborn hamsters, attempts to encourage foster-mothering were abandoned. This necessitated a change in the protocol to ascertain the mode of transfer of maternal antibodies from mother hamsters to their offspring. Alternatively, near-term females were delivered by caesarian section and blood was collected from the prenates, largely from severed vessels. This method of blood collection was much less than desirable as samples were highly diluted with tissue fluids. In only one instance were HI antibodies found in the blood from a litter of prenates and SDN antibodies were found in the blood of 3 other litters. In all instances the titers were low. Because of the tissue fluid dilution factor this investigator feels that the presence of these antibodies provides little other than qualitative evidence of the intrauterine transfer of maternal antibodies from mother hamsters to their offspring. The significance of these findings, however, is that they show that the hamster is similar to other rodent species in that, at least some, antibody transfer occurs between mother and young <u>in utero</u>.

Further studies were made to determine if maternal antibodies were transferred by way of the colostrum and milk and to ascertain if maternal antibodies protected neonatal hamsters against infection with lethal doses of TC-83 VEE virus. Newborn hamsters of VEE immune mothers were challenged with 1,000 SHipLD$_{50}$ ($10^{2.5}$PFU) of TC-83 VEE virus. The survival time for these animals was increased significantly over that for neonates born to non-immune mothers. The increased survival of the former groups was attributed to the presence of specific antibodies against VEE virus. The results provided unequivocal evidence that newborn hamsters had received specific VEE antibodies from their mothers and that these antibodies were protective against lethal infection with VEE virus. It had been shown in a previous study that hamsters transfer some antibodies to foeti in utero, but it had not been possible to quantitate these antibodies. Thus it could not be stated unequivocally that the total complement of maternal antibodies was received by foeti by intrauterine transfer mechanisms. However, the evidence strongly suggested that this did not occur. Blood samples from a large number of prenatal litters of hamsters were tested with negative results, whereas samples from challenged young as long as 21 days after challenge (25 days after birth) were positive. No evidence was obtained to suggest that these post-challenge antibodies resulted from immunization by the challenge virus. The investigator, therefore, feels that they were passively acquired maternal antibodies. He does not consider the tissue fluid dilution factor to have been so high in the prenatal samples as to prevent the detection of antibodies in a larger proportion of the litters had they been present in protective titers. On the basis of these findings and reasonings the conclusion was drawn that hamsters, like other rodents, receive passive maternal antibodies from their mothers both in utero and, post-natally, through the colostrum and milk.

Next, studies were conducted to determine if pre-existing Group A arbovirus antibodies influenced the humoral antibody response to heterologous Group A arbovirus vaccines. Studies were also made to determine if the humoral antibody response elicited by the simultaneous inoculation of 2 Group A arbovirus vaccines differed significantly from the responses resulting after the administration of the vaccines separately. The TC-83 VEE and the Clone-15 WEE vaccines were used in these studies. The findings were similar to those to be reported for horses by this investigator. There was a selective inhibition of HI or SDN antibodies, depending upon the pre-existing titer of the specific type (HI or SDN) of antibodies. The Clone-15 WEE vaccine appeared to be more effective as an immunogen than the TC-83 VEE vaccine, but this might have been related more to vaccine virus dose than to inherent vaccine efficacy. The antigenic stimulus, with respect to numbers of infectious virus particles, was greater for the Clone-15 vaccine than for the TC-83 vaccine. This was because of the increased lethality of the TC-83 VEE virus when administered in doses greater than $10^{2.5}$ PFU. The dose of Clone-15 WEE virus was on the order of $10^{7.72}$ PFU. It was noteworthy that although the hamsters gave humoral antibody responses against both the VEE and WEE vaccines administered simultaneously the responses were different with respect to antibody type. VEE SDN antibody titers were high but VEE HI antibody titers were low. Conversely, the WEE vaccine elicited high HI, but low SDN, antibody responses. The cause(s) for these phenomena could not be determined from the data. TC-83 virus was more highly infectious and/or lethal than Clone-15 virus. This was indicated by the fact that a mortality rate in excess of 20 percent was obtained with $10^{4.7}$ PFU of TC-83 virus while no deaths occurred in hamsters inoculated with $10^{7.72}$ PFU of Clone-15 virus. It is possible that whatever factor(s) were responsible for the increased infectivity/lethality were also responsible for the induction of greater VEE SDN antibody response.

The findings in these studies lead to the conclusion that the hamster is not an ideal experimental animal species to use as a model to study the immunology and epidemiology of VEE virus infections. Although this species, in many respects, responds to VEE virus infections similar to equines there are some significant differences. The most serious of these differences relate to the maternal behavior of hamsters and especially that observed after inoculation with TC-83 virus. Such behavior was responsible for the loss of many newborn hamsters through cannibalism.

Immunologically, the hamster was found to be similar to other rodents with respect to the mode of transfer of antibodies from mothers to young. Because of this there is no advantage in selecting this animal for immunological studies over other common laboratory rodent species such as the Swiss mouse. This latter species has been shown to transmit maternal antibodies to the newborn in utero and in the milk for several days after birth. Moreover, such antibodies have been demonstrated to protect neonatal mice against infection with the inducing viruses (Iida, et al., 1973; Kiuchi, et al., 1973). The maternal behavior of mice is such that these animals can be induced to foster-rear foreign young, and Zarate (1971) reported that mice survive infections with TC-83 VEE virus and respond to these infections with the production of humoral antibodies. Other epidemiolgical characteristics of the TC-83 virus, such as the capacity to infect by way of contact and/aerosol transmission, have not been worked out for this species. However, the available knowledge suggests that the Swiss mouse would be a better species for studies on VEE virus infections than the Syrian hamster.

Table 1.

HI antibody response in hamsters subsequent to vaccination with TC-83 VEE vaccine

Titer	VEE	WEE	EEE
<10 [a]	2 [b]	16	16
10	0	0	0
20	4	0	0
40	4	0	0
80	1	0	0
160	4	0	0
320	1	0	0
>640	0	0	0
Total	16	16	16
Gm titer	1:59	<1:1	<1:1
Md titer	1:40	<1:10	<1:10

Vaccine dose = $10^{4.7}$ Pfu

Mortality = 24%

a. Reciprocal of serum titer.
b. Number of animals at titer.

Table 2.

HI and SDN antibody titers[a] in hamsters subsequent to vaccination with TC-83 VEE vaccine.

Hamster No.	HI		SDN	
	Pre-vac	Post-vac	Pre-vac	Post-vac
604	<10	<10	<10	<10
606	<10	<10	<10	40
610	<10	80	<10	1,280
633	<10	40	<10	80
634	<10	<10	<10	10
636	<10	<10	<10	<10
640	<10	20	<10	320
642	<10	40	<10	640
644	<10	20	<10	160
653	<10	20	<10	320
654	<10	10	<10	<10
663	<10	<10	<10	<10
637	<10	20	<10	80
638	<10	320	<10	320
Total	14	14	14	14
Gm titer	<1:1	1:40	<1:1	1:218
Md titer	<1:10	1:20	<1:10	1:80

Vaccine dose = $10^{2.5}$ Pfu

Mortality = 10%

a. Reciprocal of serum titer.

Table 3.

HI antibody response in hamsters subsequent to vaccination with Clone-15 WEE vaccine

Titer	VEE	WEE	EEE
<10[a]	11[b]	0	11
10	0	0	0
20	0	0	0
40	0	0	0
80	0	1	0
160	0	1	0
320	0	4	0
≥640	0	5	0
Totals	11	11	11
Gm titer	<1:1	≥1:363	<1:1
Md titer	<1:10	1:320	<1:10

Vaccine dose = $10^{7.72}$ Pfu

Mortality = 8%

a. Reciprocal of serum titer.

b. Number of animals at titer.

Table 4.

Survivorship and antibody status of newborn hamsters subsequent to challenge with 1,000 LD_{50} of TC-83 strain VEE virus

Hamster No.	Mother's VEE titers[a] at time of challenge		Newborn hamsters surviving > 6 days post-chal.	VEE titers in newborn hamsters 22 days post-chal.	
	HI	SDN		HI	SDN
603	20[a]	1,280	0/2	-	-
604	40	10	0/5	-	-
613	40	5,120	3/3	<10	<10
638	160	5,120	10/10	<10	5,120
649	320	10,240	4/4	40	<10
661	40	5,120	0/5	-	-
663	20	5,120	7/7	<10	20
664	10	20	0/8	-	-
669	40	1,280	6/6	<10	<10
Controls	10	10	0/10	-	-

[a] Reciprocal of serum titer

Table 5.

VEE and WEE antibody responses in Clone-15 WEE vaccinated hamsters at 0, 14, and 28 days following cross immunization with TC-83 VEE vaccine (California, 1972)

Hamster No.	WEE D + 0 HI	WEE D + 0 SDN	WEE D + 14 HI	WEE D + 14 SDN	WEE D + 28 HI	WEE D + 28 SDN	Hamster no.	VEE D + 0 HI	VEE D + 0 SDN	VEE D + 14 HI	VEE D + 14 SDN	VEE D + 28 HI	VEE D + 28 SDN
831	160[a]	10	≥ 640	<10	320	<10	831	<10	<10	160	320	80	320
832	320	<10	≥640	<10	320	<10	832	20	80	40	80	20	80
839	80	10	160	10	320	<10	839	<10	<10	<10	<10	<10	<10
840	80	<10	320	<10	320	10	840	<10	<10	80	320	40	160
842	160	10	≥640	<10	320	<10	842	<10	<10	40	320	40	80
Gm titer	1:139	1:10	≥1:422	1:10	1:320	1:10	Gm titer	1:20	1:80	1:67	1:226	1:40	1:135
Md titer	1:160	1:10	≥1:640	<1:10	1:320	<1:10	Md titer	<1:10	<1:10	1:40	1:320	1:40	1:160

a. Reciprocal of serum titer.

Table 6.

VEE and WEE antibody responses in TC-83 immunized hamsters at 0, 14, and 28 days following cross-immunization with Clone-15 WEE vaccine (California, 1972)

Hamster No.	VEE D + 0 HI	VEE D + 0 SDN	VEE D + 14 HI	VEE D + 14 SDN	VEE D + 28 HI	VEE D + 28 SDN	Hamster no.	WEE D + 0 HI	WEE D + 0 SDN	WEE D + 14 HI	WEE D + 14 SDN	WEE D + 28 HI	WEE D + 28 SDN
811	40[a]	160	-	-	-	-	811	<10	<10	-	-	-	-
813	80	640	80	160	80	160	813	<10	<10	80	<10	160	<10
815	80	160	160	320	80	160	815	<10	<10	160	10	80	<10
816	<10	<10	<10	<10	<10	<10	816	<10	<10	160	10	160	<10
817	20	2,560	-	-	320	320	817	<10	<10	-	-	320	10
820	20	40	80	160	160	160	820	<10	<10	160	<10	<10	<10
821	160	640	160	1,280	160	640	821	<10	<10	160	<10	160	10
825	80	80	80	160	80	160	825	<10	<10	160	10	160	<10
828	80	320	160	320	160	1,280	828	<10	<10	160	<10	160	<10
Gm titer	1:57	1:269	1:131	1:285	1:113	1:290	Gm titer	<1:10	<1:10	1:160	1:10	1:142	1:10
Md titer	1:80	1:320	1:160	1:320	1:80	1:160		<1:10	<1:10	1:160	<1:10	1:160	<1:10

Gm = Geometric mean

Md = Median

a. Reciprocal of serum titer.

Table 7.

VEE and WEE antibody responses in hamsters at 0, 14, and 28 days following being simultaneously vaccinated with TC-83 VEE- and Clone-15 WEE- vaccines
(California, 1972)

Hamster No.	VEE D + 0 HI	VEE D + 0 SDN	VEE D + 14 HI	VEE D + 14 SDN	VEE D + 28 HI	VEE D + 28 SDN	Hamster no.	WEE D + 0 HI	WEE D + 0 SDN	WEE D + 14 HI	WEE D + 14 SDN	WEE D + 28 HI	WEE D + 28 SDN
TPM 1	<10[a]	<10	40	320	40	160	TPM 1	<10	<10	160	10	320	<10
TPM 2	<10	<10	20	320	40	160	TPM 2	<10	<10	40	<10	320	<10
TP 3	<10	<10	160	640	80	1,280	TP 3	<10	<10	80	<10	≥640	10
TP 4	<10	<10	<10	<10	<10	<10	TP 4	<10	<10	320	10	320	20
845	<10	<10	<10	<10	<10	<10	845	<10	<10	160	10	≥640	<10
846	<10	<10	<10	<10	<10	<10	846	<10	<10	160	10	320	10
847	<10	<10	160	320	20	160	847	<10	<10	320	<10	640	10
849	<10	<10	40	320	40	640	849	<10	<10	640	20	640	10
Gm titer	<1:10	<1:10	1:61	1:368	1:40	1:320	Gm titer	<1:10	<1:10	1:1	1:11	≥1:452	1:11
Md titer	<1:10	<1:10	1:40	1:320	1:40	1:160	Md titer	<1:10	<1:10	1:160	1:10	1:320	1:10

a. Reciprocal of serum titer.

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