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Author: McLEAN, ROBERT G.

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POTENTIATION OF KEYSTONE VIRUS INFECTION IN COTTON RATS BY GLUCOCORTICOID-INDUCED STRESS[1]

ROBERT G. McLEAN, Vector-Borne Diseases Division, Bureau of Laboratories, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Post Office Box 2087, Fort Collins, Colorado 80522, USA.

Abstract: Cotton rats *(Sigmodon hispidus)* were treated daily with corticosteroids and then inoculated with Keystone virus. Viremia and neutralizing antibody profiles were determined in treated and untreated rats. Compared to untreated rats, the treated rats were substantially more susceptible to infection, and their viremia lasted much longer. This experimental model suggests that stress associated with excess glucocorticoid synthesis within a natural population could cause an increase in transmission of arboviruses. It also suggests that the effects of stress should be considered when experimental laboratory studies are designed.

INTRODUCTION

The susceptibility to pathogens may change when animals are subjected to conditions of stress. Most stressors cause an increase in susceptibility, (Boring et al., 1956; Davis, 1960; Davis and Read, 1958; Enright et al., 1970; Friedman et al., 1969; Jackson and Farmer, 1970; Noble, 1961) although with some nutritional stresses susceptibility is reduced (Scrimshaw et al., 1968). There are a large number and variety of effective stressors as well as a variety of pathways through which stress can directly (Subba Rao and Glick, 1970) or indirectly (Barrett and Stockham, 1963; Burns et al., 1960; Chang and Rasmussen, 1965; Christian and Davis, 1964; Jackson and Farmer, 1970) act to alter an animal's susceptibility.

The nature of the relationship between stress and susceptibility to pathogens varies with the type and degree of stress and with the type and quantity of the antigen to which the animal host is subjected, (Enright et al., 1970) as well as with the timing of the administration of the stress and of the exposure to the antigen (Jackson and Farmer, 1970; Lebetkin, 1974). Further, there are species differences in responses to stress (Bronson and Eleftheriou, 1963).

Some of the indirect pathways for stress to alter susceptibility involve a pituitary-adrenal response; the adrenocortical hormones (primarily glucocorticosteroids) that are produced by the stressed animals, suppress their own immune (Bhatt and Jacoby, 1976; Bronson and Eleftheriou, 1963; Christian, 1978; McMaster and Franzl, 1961; Vessey, 1964; Wertheim and Giles, 1971) and nonimmune (Chang and Rasmussen, 1965; Subba Rao and Glick, 1970) host defense mechanisms. The adrenocortical hormones, in general, cause an involution of lymphoid tissue (Dukor and Dietrich, 1968) and thus interfere with cell-mediated immunity, and they also interfere with the synthesis of humoral antibody (Lebetkin, 1974; McMaster and Franzl, 1961; Soave and Fuenzalida, 1969; Wertheim and Giles, 1971) and suppress the production of

[1] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

interferon (Chang and Rasmussen, 1965). These mechanisms play a significant role in determining the outcome of acute infections. Other mechanisms are capable of inducing metabolic and chemical changes similar to those induced by elevated corticosteroids, but they are not well defined.

Most of the studies of the effect of stress on infections have investigated inducing stress in animals in laboratory situations by manipulating the physical interactions of the animals (crowding, fighting, etc.) (Davis and Read, 1978; Jackson and Farmer, 1970; Lebetkin, 1974; Vessey, 1964; Wertheim and Giles, 1971), by subjecting the animals to external stressors (sound, temperature, etc.) (Boring et al., 1956; Carmichael et al., 1969; Chang and Rasmussen, 1965; Friedman et al., 1969; Subba Rao and Glick, 1970) by changing the nutritional levels of the animals (Nathan et al., 1977; Scrimshaw et al., 1968), or by chemically blocking or suppressing natural defense mechanisms (Applegate, 1970; Bhatt and Jacoby, 1976; Burns et al., 1960; Camenga et al., 1974; Enright et al., 1970; Soave and Fuenzalida, 1969).

The purpose of this study was to investigate the effect of stress on a virus-host relationship, Keystone virus and cotton rats *(Sigmodon hispidus),* that could be readily manipulated in the laboratory. Keystone virus, a member of the California group of arboviruses, naturally infects cotton rats in southeastern United States (Taylor et al., 1971) and is transmitted between hosts by *Aedes* mosquitoes (Taylor et al., 1971; Watts et al., 1979). Investigation of the effects of stress on this virus-host relationship could provide information useful in the understanding of other arthropod-borne virus infections of vertebrate animals. This paper will report on the effect of chemically induced stress on the course of infection and antibody response of laboratory-reared cotton rats to Keystone virus.

MATERIALS AND METHODS

Four- to five-week-old cotton rats were obtained from a laboratory colony (established from wild rats captured in southern Georgia 12 years ago) maintained at the Lawrenceville, Georgia, facility of the Centers for Disease Control. After being shipped to Fort Collins, Colorado, they were kept individually in $52 \times 23 \times 23$ mm solid cages for 2 months before the experiment; this allowed them to adjust to the higher elevation. They were given standard laboratory rat chow and water *ad lib* and were maintained at approximately 22 C and 45% RH. There were four experimental groups of eight cotton rats each (four of each sex) and three control groups of five cotton rats each.

Approximately 250 Vero cell plaque-forming units (PFU) of KEY (WX) virus (originally isolated from *Aedes atlanticus-tormentor* collected in 1963 from Waycross, Georgia) at the 3rd suckling mouse passage was inoculated subcutaneously (sc) into rats in the four experimental groups. Rats in experimental groups I and II were also inoculated sc with 1 mg (6.1 mg/kg body weight) of a synthetic adrenal hormone (prednisolone butylacetate) every other day for20 days starting 10 days before being inoculated with KEY virus. Prednisolone has primarily glucocorticoid potency, and its anti-inflammatory effect is estimated to be four times that of cortisone (Jailer, 1959). Rats in experimental groups III and IV were inoculated on the same schedule with an equal volume of phosphate buffered saline (PBS). The rats in groups I and III were bled daily for the first 10 days, and rats in groups II and IV were bled every other day to reduce handling stress.

Three groups of control animals were used. Rats in group V had preexisting neutralizing antibody to KEY; they had been inoculated with KEY virus and were viremic 6 months prior to use in this experiment. These rats were treated with prednisolone on the same schedule as the

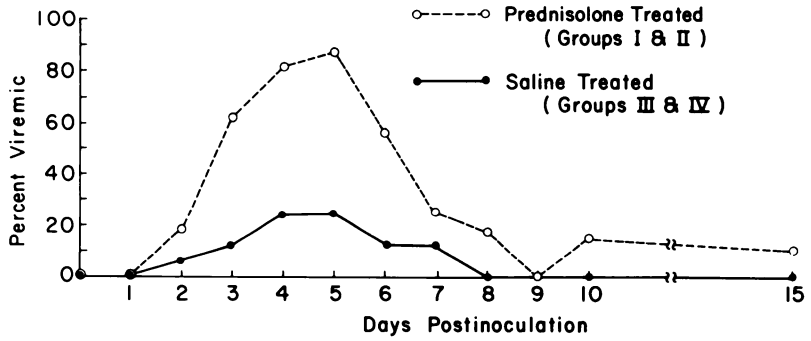
rats in groups I and II and bled every six days starting 10 days before virus inoculation of experimental rats. The nonimmune control rats in group VI were handled similarly and were inoculated only with PBS, nonimmune rats in group VII were inoculated with prednisolone, and both groups were bled on the same schedule as group V.

Blood (0.2 ml) was obtained from the orbital sinus by using a capillary pipet; the blood was discharged into 0.9 ml of diluent (Medium 199 with 20% fetal calf serum (FCS) containing penicillin and streptomycin). The diluted serum, separated by centrifugation, was stored in vials at -70 C until tested. For virus titration, serial tenfold dilutions of the sera were prepared in diluent and a 0.1 ml volume of each dilution was inoculated onto monolayers of Vero cells in 25 cm^2 flasks. After 1 h absorption at 37 C, the cultures were overlaid with nutrient medium containing 1% Noble* agar. After incubation at 37 C for 2 days, a second overlay containing 1:25,000 neutral red was added. The cultures were incubated for another day, and plaques were counted.

For neutralizing antibody tests, sera were inactivated at 56 C for 30 min and mixed with equal volumes of KEY virus diluted to contain 100 PFU. After 1 h of incubation at 37 C, 0.1 ml of the mixture was added to Vero cell cultures, and the inoculated cultures were handled as described above. Reduction of 90% or more of plaques was considered positive for KEY neutralizing antibody. Sera positive in the first test were serially diluted twofold, starting at 1:10, to determine antibody titers. The analysis of variance, Student's t-test, and Chi-square test were used to analyze the data and compare experimental groups.

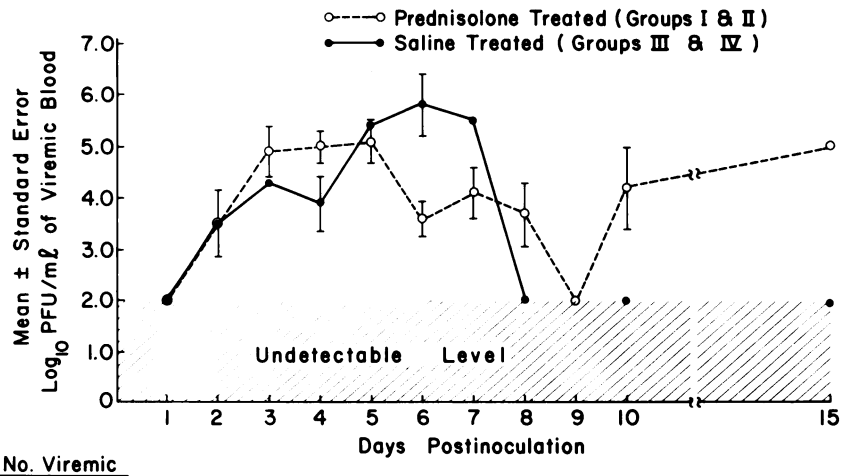
RESULTS

Prednisolone-treated rats (Groups I and II) were not only significantly ($p<.001$) more susceptible to infection (100% vs 25%), but the virus was present in the blood of prednisolone-treated rats more frequently (36% vs 8%) ($p<.001$) and for a longer time than PBS-treated rats (Groups III and IV) (Figs. 1 and 2). There was a greater proportion of viremic rats each day in the prednisolone groups (Fig. 1). Virus generally appeared in the serum



No. Viremic / No. Tested											
Prednisolone	0/8	3/16	5/8	13/16	7/8	9/16	2/8	3/16	0/7	2/13	1/10
Saline	0/8	1/16	1/8	4/16	2/8	2/16	1/8	0/16	0/8	0/16	0/15

FIGURE 1. The effect of prednisolone treatment on the percentage of cotton rats viremic for 15 days after inoculation with Keystone Virus.



No. Viremic / No. Tested											
Prednisolone	0/8	3/16	5/8	13/16	7/8	9/16	2/8	3/16	0/7	2/13	1/10
Saline	0/8	1/16	1/8	4/16	2/8	2/16	1/8	0/16	0/8	0/16	0/15

FIGURE 2. The effect of prednisolone treatment on the mean titer of Keystone Virus in cotton rats.

at the same time postinoculation (PI) in prednisolone and PBS groups; however, three of the 16 viremic rats in the prednisolone groups had delayed viremias, starting after 9 days PI. Treatment had no significant effect on the mean titer of virus in all rats on all viremic days (4.48 ± .17 vs 4.67 ± .32 $\log_{10}$PFU/ml ± SE) nor on the mean peak titers for individual animals. The mean viremia titers of rats in prednisolone and PBS groups were similar (Fig. 2) only during the first 5 days PI. On day six, peak viremia was observed in the PBS-treated rats, whereas viremia titers in prednisolone-treated rats had declined. No viremias were detected in PBS rats after day seven. Virus was present after day nine at high titers in three prednisolone-treated animals with delayed onset of viremia.

There was no significant effect due to bleeding schedule or sex. The mean titers of animals bled daily (Groups I and III) appeared higher than titers for those bled every other day (Groups II and IV). The mean titers of animals in Groups I and II (4.79 ± .21 vs 3.93 ± .26 $\log_{10}$PFU/ml ± SE) were significantly different ($p<.02$); however, if only those sera from every other day are compared for all groups, then there is no significant difference.

Neutralizing antibody was found only in cotton rats which had a detectable viremia. No antibody was detected in rats in control groups VI and VII kept in the same room with KEY-infected rats. In rats with neutralizing antibody, the logarithm of geometric mean titers (GMT) of antibody were significantly higher ($p<.05$) in PBS-treated than prednisolone-treated rats (2.62 ± .18 vs 2.20 ± .08 log GMT ± SE). The antibody response in prednisolone-treated rats was delayed, and the titers remained at a lower level than those for PBS-treated rats (Fig. 3). Although the overall neutralizing antibody titers were significantly different between PBS- and prednisolone-treated rats, there were no statistically significant differences on any individual days. The log GMT of

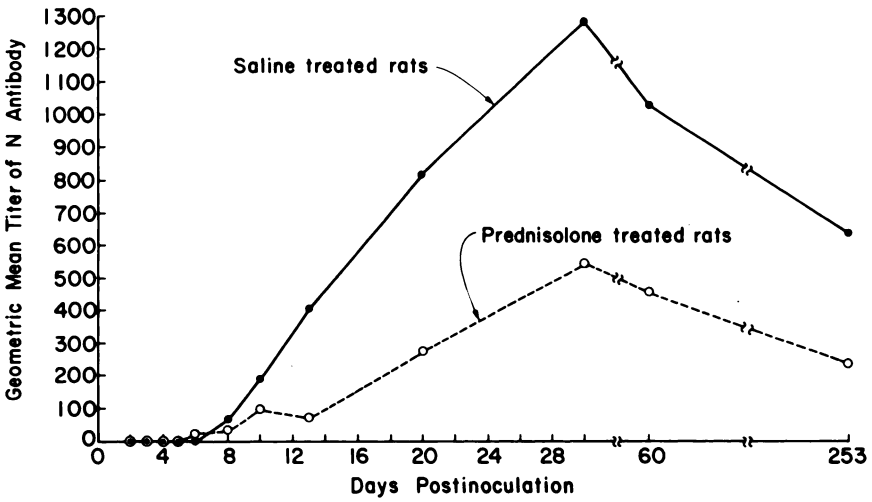


FIGURE 3. Geometric mean titers of neutralizing (N) antibody following inoculation with Keystone Virus in Prednisolone- and saline-treated cotton rats.

preexisting KEY antibody in the positive control rats was depressed from 1.28 (± .34 SE) or 19 GMT before treatment with prednisolone to nondetectable levels within 2 days after the first injection. After 10 days of prednisolone, the log GMT increased to 1.68 (± .15 SE) and during the next 14 days declined to the same level as the preexisting log GMT of antibody. The log GMT gradually increased until it peaked at 1.90 (± .18 SE) 70 days after the first prednisolone injection.

There was a significantly higher mortality ($p<.02$) in the prednisolone-treated, KEY rats (44%) in Groups I and II than in PBS, KEY rats (6%) in Groups III and IV; however, neither were significantly different from that for the prednisolone-treated, control rats (Group VII), since 20% (1/5) died within 30 days PI. There was no attempt to examine the histologic changes associated with the mortalities. The specific cause of death was not determined.

DISCUSSION

The results indicate that the physiologic status of an animal established as a result of stress, chemically induced in this study, has a profound effect on the ability of an animal to resist a virus challenge. Cotton rats are normally only moderately susceptible to KEY virus, i.e., 25% became infected after being inoculated with the virus experimentally. The magnitude and duration of viremia in cotton rats in this study were similar to the results obtained from gray squirrels *(Sciurus carolinensis)* and cottontail rabbits *(Sylvilagus floridanus)* inoculated with another strain of KEY virus (Watts et al., 1979). The squirrels and rabbits were highly susceptible to KEY virus, but the dose of virus inoculated into cotton rats in this study was much lower which would account for the moderate susceptibility in the PBS-treated cotton rats. Increased levels of corticosteroids (prednisolone treatment) significantly enhanced the susceptibility of cotton rats to KEY virus, as it did with other viruses and other vertebrate species (Bhatt and Jacoby, 1976; Enright et al., 1970).

Prednisolone apparently affected only the synchrony of the viremic response among rats in the treatment groups and

not the magnitude of the response, since there was no significant difference in virus titers among prednisolone and PBS-treated rats. The mechanisms responsible for increasing the intrinsic incubation period and delaying onset of viremia in some of the prednisolone-treated rats are not known.

Administration of corticosteroid hormone in this experimental model could have interfered with several host defense mechanisms. It is clear that the corticosteroid treatment delayed and depressed the magnitude of the neutralizing antibody response following the primary infection, but did not abrogate the response as in the case of cyclophosphamide in mice infected with West Nile virus (Camenga et al., 1974). Prednisolone treatment temporarily suppressed circulating antibody in KEY immune cotton rats as in rabies-immune mice (Soave and Fuenzalida, 1969). The cotton rats with preexisting antibody were not rechallenged with KEY virus to determine if they were susceptible after being treated with prednisolone. The antibody level in this group of rats, however, increased beyond the pretreatment level after prednisolone treatment ceased. This could have resulted from increased synthesis of antibody after release from suppression or from an amnestic response to recirculation of virus. The effect of prednisolone on other mechanisms such as cell-mediated immunity and interferon production were not measured, and interference with these mechanisms could have also contributed greatly to the change in susceptibility to KEY virus.

Since naturally produced corticosteroids have a similar effect upon the susceptibility of rodents to infection (Boring et al., 1956; Davis, 1960; Davis, 1978; Davis and Read, 1958; Jackson and Farmer, 1970; Noble, 1961), stress-potentiated infections are believed to play a density-dependent role in regulating populations of some vertebrate species (Christian, 1978; Christian and Davis, 1964; Davis, 1978; McLean, 1975). The impact of altered natural resistance in vertebrate hosts on the transmission of pathogens is, however, of even greater concern. Stress in host populations produced through overcrowding, environmental disturbance, nutritional deficiencies and other stimuli could favor amplification of pathogen transmission if other factors, such as the presence and synchrony of pathogen populations and vector populations are suitable. The results from this study demonstrated that substantially more viremic cotton rats would be available to infect vector mosquitoes, and mosquitoes could become infected over a much longer period of time. This indicates that factors other than the number of susceptible hosts could be important in determining the contribution of a particular host species to disease transmission. The interaction between infected hosts and susceptible vectors could be greatly expanded during conditions of stress, and if vector populations are sufficiently abundant and active, transmission rates could be increased substantially. In addition, such a mechanism timed with natural, seasonal changes in environmental stress in a host population and with the emergence of mosquitoes could be important in the annual maintenance cycle for arboviruses as it is for bird malaria (Applegate, 1970; Beaudoin et al., 1971).

The results from this experiment also indicate that scientists conducting laboratory studies should consider and control the effects of stress on the response of vertebrate animals to experimental infection. Results from studies in which the influence of stress on the susceptibility and response of host animal species is ignored and not measured should be viewed with caution. Such results should not be regarded as representing the normal response of vertebrates to natural infections.

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