15826

Susceptibility of the Gray Fox to Fox Encephalitis.

R. G. GREEN AND C. S. STULBERG.

From the Department of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis.

Studies on the adaptation of the virus of fox encephalitis to animal species have shown that this virus can invade related groups of animals with varying degrees of pathogenicity. In members of the family Canidae, such as the red and silver foxes, dogs, and coyotes,[1] experimental infection with the virus results in a high mortality, while such species as the black bear[2] and the raccoon,[3] which are offshoots of the canines, are only slightly susceptible.

The gray fox (Urocyon) is only distantly related to the red fox group (Vulpes). The latter is related to European forms, whereas the gray fox is probably a survivor of American preglacial fauna and appears to be more closely related to the South American foxes and dogs. In view of these relationships, it has been of interest to study the susceptibility of gray foxes to fox encephalitis.

Our earliest experiments[1] indicated that gray foxes inoculated with the virus by cisterna puncture did not show evidence of infection. In a later experiment one of 2 gray foxes inoculated by simultaneous cisterna puncture and intramuscular injections developed symptoms of fox encephalitis and was killed at the end of 4 days. Microscopic examination revealed characteristic inclusion bodies in the meninges and brain capillary endothelium. Inclusions were also seen in hepatic and reticular-endothelial cells of the liver and in the endothelium of the kidney glomeruli. More recently we have been able to show that a symptomless infection can be produced regularly in gray foxes and that only occasionally will an infection result in death.

Attempts were made to infect gray foxes by intracerebral, intraperitoneal, or intraocular injections. Such inoculations in red foxes result in an acute systemic infection with a high mortality.[4,5] Endothelial cells of organs infected, particularly the endothelial cells of the brain capillaries, show intranuclear inclusion bodies which are characteristic of the disease.[6] When the virus is inoculated into the anterior chamber of the eye, the single layer of endothelial cells lining the posterior surface of the cornea is infected. This is indicated by the presence of inclusions and the development of a corneal opacity.[5]

The virus regularly used in our experimental work is a homogenized suspension of infected fox brain preserved as 20% tissue in 50% neutral glycerin. The source of each of virus lots 77, 88, and 98 were pooled brain suspensions from not less than 10 infected red foxes which were allowed to die from the infection or were killed *in extremis*. These pooled viruses were subsequently tested in red foxes and were found to be highly virulent.

In a preliminary experiment the 20% suspension of pooled lots 77, 88, and 98 was diluted to a 2% suspension in Ringer's solution. 0.1 cc was injected into the anterior chamber of the left eye of an 8-weeks-old gray fox pup. In 3 days the eye was completely opaque. The animal was killed, and smears of the corneal endothelium showed

1 Green, R. G., Ziegler, N. R., Carlson, W. E., Shillinger, J. E., Tyler, S. H., and Dewey, E. T., *Am. J. Hyg.*, 1934, **19**, 343.

2 Stulberg, C. S., and Green, R. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 88.

3 Green, R. G., Evans, C. A., and Yanamura, H. Y., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 186.

4 Green, R. G., Ziegler, N. R., Dewey, E. T., and Shillinger, J. E., *Am. J. Hyg.*, 1931, **14**, 353.

5 Evans, C. A., Yanamura, H. Y., and Green, R. G., PROC. SOC. EXP. BIOL. AND MED., **1943, 53**, 183.

6 Barton, J. C., and Green, R. G., *Am. J. Hyg.*, 1943, **37**, 21.

typical intranuclear inclusions. The aqueous humor was bacteriologically sterile. No inclusion bodies were observed in the brain capillaries. Presence of the virus in the brain of the gray fox was shown by aseptically removing a portion of the cerebellum, which was then ground with mortar and pestle and diluted to a 5% suspension with Ringer's solution. 0.1 cc was injected into the anterior chamber of the eye of the highly susceptible red fox, which developed a corneal opacity in 4 days and died of typical fox encephalitis in 5 days.

0.25 cc of 20% pooled virus from lots 77, 88, and 98 was then inoculated intracerebrally into two 8-weeks-old gray fox pups. At the end of 5 days these animals had not developed symptoms, although with red foxes a 2- to 4-day incubation period was commonly observed under similar conditions. The gray foxes were killed and their brains and livers were removed aseptically. Microscopic examination revealed no evidence of infection. Equal parts of the brains were pooled and the mixture diluted to 20% in Ringer's solution. This was designated "gray fox passage virus" and was inoculated into a second group of 4 young gray fox pups as follows: (1) 0.25 cc intracerebrally and 0.1 intraocularly into the anterior chamber of the left eye: (2) 0.25 cc intracerebrally; (3) and (4) 0.25 cc intracerebrally simultaneously with 0.25 cc of pooled 20% virus from lots 77, 88, and 98.

The first gray fox, which was inoculated with gray fox passage virus intracerebrally and intraocularly, developed a slight corneal opacity in 3 days. The opacity became progressively marked by the end of 8 days, at which time the animal appeared sluggish in its movements and would not eat. The fox was killed, and inclusions were found in the corneal endothelium but not in the brain or other tissues. A portion of the brain was removed aseptically, ground and diluted to a 5% suspension in Ringer's solution, and injected into the anterior chamber of the eye of a red fox. This animal developed a positive eye reaction in 3 days and died in 5 days with symptoms and microscopic findings of fox encephalitis. The second gray fox, inoculated with gray fox passage virus intracerebrally, was normal at the end of 5 weeks.

The 3rd and 4th gray foxes were inoculated intracerebrally with gray fox passage virus together with equal amounts of original red fox virus. One animal remained normal, while the other developed symptoms of fox encephalitis by the 8th day. This animal had occasional convulsions and exhibited a marked weakness of its hind legs. When standing up, it appeared to be in a lethargic state, but weaved back and forth unsteadily. The fox was killed *in extremis*. Numerous intranuclear inclusions were found in the brain capillary endothelium and in the liver reticular-endothelium. Material from the brain of this animal, subsequently injected intraocularly into a red fox, produced the typical eye reaction, symptoms, and death, with typical pathology of fox encephalitis.

The 2 gray foxes that remained normal in this group developed a distemper infection 5 weeks later and were discarded.

Pooled brain and liver from all the gray foxes that showed evidence of a fox encephalitis infection, together with the original pooled virulent red fox virus from lots 77, 88, and 98, were inoculated into a group of 4 young gray foxes. 0.5 cc of a 20% suspension of the pooled tissues was injected intracerebrally, simultaneously with 2 cc injected intraperitoneally. Two of the animals were sacrificed at the end of 6 days, although no symptoms had been observed. Microscopic examination of the brains of both animals revealed several typical intranuclear inclusions in the capillary endothelium. The brain material, subsequently inoculated intraocularly into red foxes, produced the characteristic eye reactions, symptoms, and death from fox encephalitis. The 2 gray foxes that were allowed to live remained normal for 5 weeks, after which time they developed a distemper infection and were discarded. When brain from one of these animals was inoculated intraocularly into a red fox, no viable virus was found.

Summary. The results indicate that the gray fox is relatively resistant to fox encephalitis virus virulent for red foxes. It appears that the virus will regularly infect the gray fox to produce a symptomless infection,

but only occasionally will symptoms appear that resemble the disease in red foxes.

A virulence gradient of this virus for related species can be postulated whereby in the family *Canidae* the closely related dogs and coyotes (*Canis*) and red foxes (*Vulpes*) are most susceptible, the more distantly related gray fox (*Urocyon*) less susceptible, and those species which are earlier offshoots of the canines, such as the black bear and the raccoon, are the least susceptible to fox encephalitis.

15827

Viability of the Rabbit Papilloma Virus.

R. G. FISCHER AND R. G. GREEN.

From the Department of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis.

Rabbit papillomatosis was described by Shope,[1] who established that the tumorous growth was due to a filterable virus. The occurrence of the rabbit papilloma seems to have been first recorded by Seton,[2] but the warty growths generally described as rabbit horns were well known even previously to hunters and naturalists. Thaddeus Surber[3] noted the growths on Kansas cottontail rabbits in 1899.

Our first studies of rabbit papillomatosis were in 1927 and since samples of rabbit horns collected by Green at that time were preserved, it is now possible to test samples for viability that have been in storage for almost 20 years. Recently we have tested a group of 6 viruses in storage from approximately 6 to 20 years.

From the reports in the literature, it has become obvious that stored papilloma tissue would remain viable for long periods of time. Shope in his original work used papilloma tissue which had been stored 106 days. Bryan and Beard[4] used papilloma tissue which had been stored for 3 years and found it to be capable of producing infections. Samples of the Shope virus in our laboratories have been stored as pieces of papillomatous tissue in 50% glycerine, refrigerated at 4°C.

The tissues were tested for viable virus by scarifying small areas of skin on the ears and flanks of domestic rabbits and applying small amounts of centrifuged tissue suspensions. In preparing material for inoculation, tissues were ground with a mortar and pestle and suspended in 9/10% saline to give a tissue suspension of about 10%. The suspension was centrifuged for one-half hour at 2000 r.p.m. The supernatant was used for the skin inoculation and was applied with a

TABLE I.
Duration of Viability of Shope Papilloma Virus.

Tissue No.	Source of tissue	Date collected	Date tested	Length of proven viability
1	Cottontail rabbit	Mar. 1927	Jan. 1947	19 years 10 mo.
2	" "	Jul. 1937	Nov. 1946	9 " 4 "
3	" "	Apr. 1938	Jun. 1946	8 " 2 "
4	" "	Feb. 1939	Jun. 1946	7 " 4 "
5	" "	Mar. 1939	Jul. 1946	7 " 4 "
6	" "	Apr. 1940	Sep. 1946	6 " 5 "

1 Shope, R. E., and Hurst, E. W., *J. Exp. Med.*, 1933, **58**, 607.

2 Seton, E. T., *Lives of Game Animals*, Vol. IV, Part II, 787.

3 Personal note to R. G. Green.

4 Bryan, W. R., and Beard, J. W., *J. Nat. Cancer Inst.*, 1941, **1**, 607.